

A QUANTITATIVE PHARMACOLOGICAL STUDY  
OF SOME PUTATIVE NEUROTRANSMITTERS IN THE  
CAROTID BODY OF THE CAT AND THE RABBIT

by

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A thesis presented for the degree of Ph.D.  
University of Edinburgh  
1980



## ABSTRACT

Afferent chemoreceptor activity was recorded from the peripheral cut end of the sinus nerve in anaesthetized cats and rabbits. It was found that intracarotid injection of dopamine inhibited spontaneous chemoreceptor activity in both species. 5-Hydroxytryptamine evoked a brief excitation followed by inhibition of discharge in both species. In cats, apomorphine caused a prolonged inhibition of chemoreceptor activity. Following administration of  $\alpha$ -flupenthixol or haloperidol in cats and  $\alpha$ -flupenthixol in rabbits, dopamine no longer evoked an inhibitory response but instead tended to cause an increase in chemoreceptor discharge. Responses to the chemoreceptor stimulants sodium cyanide and hypoxia were potentiated following administration of dopamine-blocking agents. These results suggest the possibility that endogenous dopamine acts to depress afferent carotid chemoreceptor activity.

Subsequent experiments in cats showed that the dopamine-uptake blockers benztropine and nomifensine, but not the monoamineoxidase inhibitor pargyline, potentiate the inhibitory action of injected dopamine and also potentiate chemoreceptor responses to sodium cyanide and hypoxia. These results imply that, in addition to its inhibitory action, endogenous dopamine has an excitatory action on chemoreceptor activity. It is suggested that the most likely physiological role for dopamine in the carotid body is as a chemical mediator in an 'amplification' system, modulating activity in sensory nerve endings which are themselves the chemoreceptors.

Injection of acetylcholine in rabbits caused inhibition of afferent chemoreceptor activity, in contrast to the stimulation evoked in cats and dogs. In some experiments high doses of

acetylcholine evoked a slight excitation which preceded the inhibition. This effect was probably brought about by an action on nicotinic receptors since it was blocked by mecamylamine but is unlikely to be of physiological significance. The muscarinic agonists methacholine and bethanechol, but not the nicotinic agonist suberyldicholine, also inhibited spontaneous chemoreceptor discharge indicating that the inhibition was brought about by an action on muscarinic receptors. The inhibition was blocked by atropine, although high doses were required to produce this effect. The inhibition is unlikely to be a consequence of a vascular action of acetylcholine since the vasodilator drugs sodium nitrite and sodium nitroprusside had little effect on chemoreceptor activity. The inhibition evoked by acetylcholine was not reduced by  $\alpha$ -flupenthixol and is, therefore, unlikely to be secondary to dopamine release. It is proposed that acetylcholine may act as a transmitter in an inhibitory efferent pathway to the carotid body but is unlikely to act as an excitatory sensory transmitter.

### DECLARATION

I declare that all the work presented in this thesis is entirely my own with the following exceptions:

1. In 12 experiments with cats surgical preparation of animals was performed partially or totally by Dr. D.S. McQueen.
2. Estimations of  $pO_2$ ,  $pCO_2$  and pH in samples of arterial blood were performed by Mr. C. Calder, Mr. K. Bell or Mrs. S. Bond.

Reg. J. Docherty



### ACKNOWLEDGEMENTS

I am indebted to Dr. D.S. McQueen for his conscientious supervision of my work and for his advice and guidance.

I would also like to thank the staff and students of the Department of Pharmacology and the M.R.C. Brain Metabolism Unit for their friendship and stimulating conversation and in particular Mr. C. Calder, Mr. K. Bell and Mrs. S. Bond for their invaluable assistance, cheerfulness and encouragement. My thanks are due to the Medical Research Council for financing my work and to Professor E.W. Horton for allowing me the use of his department and his many other favours.

A special 'thank you' also to my parents and to Maureen, my fiancée, for their love, patience and support.

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## LIST OF ABBREVIATIONS

The abbreviations listed below are used at various points in the text but not in headings. Some of the abbreviations are introduced in the text and are listed here only for convenient reference.

ACh	-	acetylcholine
ADR	-	adrenaline
BCh	-	bethanechol
B.P.	-	arterial blood pressure
cAMP	-	cyclic adenosine monophosphate
cm	-	centimetre
C.N.S.	-	central nervous system
COMT	-	catechol-O-methyltransferase
DA	-	dopamine
d.c.	-	direct coupled
DCI	-	dichloroisoprenaline
DCV	-	dense cored vesicle
DFP	-	di-isopropylfluorophosphate
DHE	-	dihydro- $\beta$ -erythroidine
D-TC	-	D-tubocurarine
g	-	gramme
HC-3	-	hemicholinium-3
HCl	-	hydrochloric acid
5-HT	-	5-hydroxytryptamine (serotonin)
Hz	-	hertz
I.C.	-	intracarotid
I.P.	-	intraperitoneal
I.V.	-	intravenous

KCN	-	potassium cyanide
kg	-	kilogramme
LSD	-	lysergic acid diethylamide
MAO	-	monoamineoxidase
MCh	-	methacholine
mg	-	milligramme
min	-	minute
ml	-	millilitre
mm Hg	-	millimetres of mercury
mol.	-	moles
msec	-	millisecond
µg	-	microgramme
NA	-	noradrenaline
NaCN	-	sodium cyanide
ng	-	nanogramme
6-OHDA	-	6-hydroxydopamine
PBA	-	phenoxybenzamine
pmol.	-	picomole
PSA	-	post-stimulus activation
SDCh	-	suberyldicholine
sec	-	second
TEA	-	tetraethylammonium
TEPP	-	tetraethylpyrophosphate

SECTION I

GENERAL INTRODUCTION

The mammalian carotid body is an organ composed of groups of specialised cells in a complex vascular network. It is a small structure, usually located bilaterally in the vicinity of the carotid bifurcation and attached either to the internal or the external carotid arteries or to the bifurcation itself. A major function of this organ is the generation of afferent sensory discharge at a frequency dependent on the  $pO_2$ ,  $pCO_2$  and the pH of the blood perfusing it. Important cardiovascular and respiratory reflexes rely on the integrity of this 'chemoreceptor' faculty, the mechanism of which is unknown.

#### EARLY HISTORY

The history of the discovery of the carotid body and the development of early ideas concerning its function have been reviewed in great detail (Funke, 1904; Adams, 1958; Pick, 1959) and only a general treatment of the subject will be given here.

Early anatomists generally considered the carotid body to be a sympathetic ganglion. The macroscopic appearance of the organ and its proximity to the superior cervical ganglion no doubt made this seem a reasonable assumption. Studies of the microscopic structure of the carotid body led to two new interpretations of the nature of the organ. Lushka (1862) considered the carotid body to be a glandular structure while Arnold (1865) believed it to be a simple vascular glomerulus. A controversy developed which lasted for several years until Stilling (1892) combined the two ideas by suggesting that the carotid body was a highly vascular gland. Stilling emphasised the similarities between this structure and the adrenals - in particular the fact that some cells in the carotid body stained

brown with potassium dichromate as did cells in the adrenals. This idea was taken up and developed by Kohn (1900) who suggested that the chromaffine cells in the carotid body, i.e. those stained brown with potassium dichromate, were derived embryologically from nervous tissue. As a result of its supposed intimacy with the sympathetic nervous system and the ability of some of its cells to exhibit the chromaffine reaction, Kohn included the carotid body in his group of so-called paraganglia and named it the 'paraganglion intercaroticum'. Attempts to demonstrate functional similarities between the carotid body and the adrenals were generally unsuccessful, however, and several workers questioned the chromaffinity of carotid body cells (see Adams, 1958).

The work of De Castro (1926) clearly showed that the predominant innervation of the organ comes from the glossopharyngeal nerve and not the sympathetic system, which was a serious blow to the notion of the carotid body as a paraganglion. The concept was correspondingly modified to allow for paraganglionic cells derived from the parasympathetic system which were non-chromaffine thus allowing for the predominance of non-chromaffine cells in the carotid body and its innervation by the glossopharyngeal nerve (for references see Adams, 1958). By this time however, De Castro had convincingly shown that the innervation of the carotid body was sensory and not motor as had previously been supposed (De Castro, 1928). De Castro correctly surmised that the carotid body is "un organe sensorial spécial dédié à percevoir quelques modifications qualitatives du sang, plutôt qu'un appareil destinée à recevoir les variations de la pression sanguine", and so not only ascribed the carotid body a chemoreceptor function but denied it the baroreceptor function which had been supposed by



others (Drüner, 1925; Jacobvici, Nitzescu and Pop, 1928). This contribution by De Castro marks a turning point in research in this field.

Around the same time as De Castro published his classical papers on the morphology and innervation of the carotid body, J.F. and C. Heymans demonstrated chemoreceptor reflexes arising from the cardio-aortic zone (Heymans, J.F. and C., 1927). Apparently unaware of De Castro's work, C. Heymans and his co-workers investigated the possibility that the carotid bifurcation region might also be a reflexogenic zone. In a long series of papers starting in 1928 (for references, see Heymans and Neil, 1958), C. Heymans and his associates characterized the reflexes arising from the carotid bifurcation region and identified the chemoreceptor reflex with the carotid body. This work provided substantial experimental evidence for De Castro's postulate and provided a basis for modern research.

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## NEUROTRANSMITTERS IN THE CAROTID BODY

The carotid chemoreceptors respond to hypoxic, histoxic and stagnant hypoxia but not to anaemic hypoxia (Comroe and Schmidt, 1938). The insensitivity of the chemoreceptors to anaemic hypoxia suggests that their discharge frequency is dependent on carotid body blood flow but not only because of the amount of  $O_2$  the blood carries to them (see Torrance, 1968). This has led to the view (Neil, 1951) that the blood supplies the chemoreceptors with something, other than  $O_2$ , a lack of which will cause them to discharge, e.g.  $HCO_3^-$ . Alternatively, the blood carries away some substance which is produced in the carotid body and is concerned in the excitation of sensory nerve endings. The latter possibility is of particular importance to the present discussion since this suggests that chemical transmission of sensory impulses might occur in the carotid body. A number of putative neurotransmitters are present in the carotid body. Two such substances - acetylcholine (ACh) and dopamine (DA) - have been considered in this thesis, the purpose of which was to investigate, using quantitative techniques where possible, the contribution, if any, of these substances to the mechanism whereby chemoreceptor activity is initiated in the carotid body.

Several reports have appeared in the literature which either support or reject the idea that one or other of these substances

P.T.O.

acts as a chemical transmitter in the carotid body. As a background to the experimental work performed in the present study, the relevant literature for ACh and for DA and related catecholamines will be reviewed in turn. Some experiments were performed with 5-hydroxytryptamine (5-HT) and for completeness the relevant literature for 5-HT will also be reviewed.

### ACETYLCHOLINE

Schweitzer and Wright (1938) first suggested the possibility that ACh might act as a sensory transmitter in arterial chemoreceptors. They showed that neostigmine (prostigmine), an anticholinesterase drug, caused reflex stimulation of respiration in anaesthetized cats by an action on peripheral chemoreceptors and found that the chemoreceptor stimulant effects of ACh were intensified following administration of either neostigmine or physostigmine (eserine). They suggested that drugs or changes in the chemical composition of the blood which stimulate the chemoreceptors might "produce their effects by liberating ACh as a chemical intermediary". A similar suggestion was made by Winder in 1938 (see Schmidt and Comroe, 1940).

Results from several studies indicated that ACh caused a reflex stimulation of respiration by an action on peripheral chemoreceptors (Heymans, Bouckaert and Handovsky, 1935; Heymans, Bouckaert, Farber and Hsu, 1936; De Wispelaere, 1937; Philipot, 1937). Prior to this, however, a variety of nicotinic drugs other than ACh had been shown to cause reflex stimulation of respiration by an action on structures in the region of the carotid bifurcation (Heymans, Bouckaert and Dautrebande, 1931a, 1931b; Owen and Gessel, 1931; Zunz and Tremonti, 1931; Dautrebande, 1932; Mercier and Rizzo, 1932;

Dautrebande and Marechal, 1933; Mercier, 1933). Several different authors noticed the close correlation between the nicotinic cholinomimetic properties of drugs and their ability to stimulate carotid chemoreceptors (Mercier, Rizzo and Delphaut, 1934; Anichkov, 1937; Phillipot, 1937; Comroe and Schmidt, 1938). Derivatives of  $\beta$ -methyl choline which exhibit muscarinic rather than nicotinic cholinomimetic properties were found to be weak or inactive (Comroe and Starr, 1933; Phillipot, 1937; Comroe and Schmidt, 1938). De Wispelaere (1937), however, reported that the acetyl ester (methacholine) and ethyl ether of  $\beta$ -methyl choline were effective chemoreceptor stimulants. Liljestrand and Zotterman (1954) also found that methacholine was a chemoreceptor stimulant. It has since been shown, however, that the stimulant effects of methacholine are due to a weak nicotinic cholinomimetic action of the drug (McQueen, 1978).

All the experiments described above were performed on anaesthetized cats or dogs or on decerebrate cats. It is interesting to note that Věrzar, Szécsényi-Nagy, Haffter and Wirz (1937) found that intravenous (I.V.) injection of ACh, physostigmine, or neostigmine caused inhibition of respiratory movement and diaphragmatic tone in anaesthetized rabbits. This result, however, is generally overlooked in later literature.

The development of a technique for recording afferent activity in single or few fibre preparations of the carotid sinus nerve (Bronk and Stella, 1932) provided a more direct method of monitoring sensory activity arising from the carotid sinus region. Using this technique Euler, Liljestrand and Zotterman (1939) showed that I.V. injections of small doses of ammonia in anaesthetized cats caused a temporary abolition of chemoreceptor discharge without affecting

baroreceptor discharge. In these experiments, chemoreceptor stimulation produced by hypoxia, hypercapnia or I.V. injection of potassium cyanide (KCN) was reduced by ammonia while the stimulant effects of nicotine and lobeline were unaffected. Since ammonia reduced the response to 'natural' stimulation but not that to nicotine or lobeline, the authors reasoned that the latter substances must act at a site central to the chemoreceptors proper. Pointing out that chemoreceptor stimulation was brought about by "very much the same agents as cause a discharge of post-ganglionic impulses from a sympathetic ganglion", they suggested that such agents may increase afferent chemoreceptor discharge by an action on ganglion cells intercalated in the afferent pathway whereas natural stimulants such as hypoxia, hypercapnia and acid act by increasing the pH at the chemoreceptors. The authors, however, offered no suggestion as to the nature of the chemoreceptors and were unclear about the anatomical relationship between ganglion cells and chemoreceptors. This concept was later modified to allow for the possibility that ACh was released from structures located in specific chemoreceptor cells and acted directly on sensory nerve endings (Zotterman, 1944; Landgren, Liljestrand and Zotterman, 1952).

The formulation of an hypothesis implicating ACh as a sensory transmitter in the carotid body prompted several workers to investigate whether or not this might be the case. The techniques employed in such studies have been varied, however, and the results obtained often equivocal. A number of criteria must be satisfied before a substance proposed as a neurotransmitter can be accepted as such (McLennan, 1963), i.e. the substance should mimic the effects of physiological stimuli, the substance must be stored in the tissue

and released by physiological stimuli, there must be a mechanism present for the synthesis of the proposed transmitter and for its inactivation and drugs which block or enhance the effect of the proposed transmitter should similarly modify the effects of physiological stimuli. To what extent these criteria apply to sensory mechanisms is uncertain. Nevertheless, it will be convenient to discuss the literature in terms of these criteria.

#### Storage and release of acetylcholine

If chemical transmission of sensory impulses occurs in the carotid body it would be desirable to measure tissue levels of transmitter substances and to demonstrate their release during chemoreceptor stimulation. The small size and awkward location of the carotid body make this difficult, if not impossible, to do. This problem was partly overcome by the development of a technique for superfusing the carotid body with oxygenated Locke solution *in vitro* (Eyzaguirre and Lewin, 1961). Using this technique, Eyzaguirre and Koyano (1965c) studied the effects of electrical stimulation of the cat carotid body on the frequency of chemoreceptor discharge recorded from the sinus nerve. They found that stimulation of the carotid body with repeated electrical impulses of short duration (0.1 msec) caused a depression of afferent chemoreceptor discharge. On withdrawal of the stimulus there was an increase in discharge above control levels which they termed post-stimulation activation (PSA). The phenomenon of PSA was dependent on the flow rate of the superfusate, being more marked at slower rates. They concluded that PSA was due to accumulation of a chemical transmitter, released during stimulation of the carotid body, which activated sensory



nerve ends on withdrawal of the electrical stimulus. They also showed that the magnitude of the PSA was reduced after prolonged exposure to gallamine or d-tubocurarine (D-TC) and, in some experiments, the duration of the PSA was increased by physostigmine. This suggested to the authors that the transmitter was an ACh-like substance. These authors obtained similar results using direct current as the stimulus but in this case the response was dependent on the polarity of the current. These latter experiments have been criticized by Biscoe (1971) since, given a random geometry of cells in the carotid body, the direct current should be equally effective whatever the direction of current flow.

Eyzaguirre, Koyano and Taylor (1965) produced convincing evidence for release of a chemical transmitter during chemoreceptor stimulation using a modification of the above technique. Two carotid bodies were superfused in series in a stream of oxygenated Locke solution. Stimulation of the upstream preparation resulted in increased chemoreceptor activity in the downstream preparation, presumably as the result of overflow of a chemical transmitter. These 'Loewi-type' experiments were repeated by Eyzaguirre and Zapata (1968b) who further showed that the 'Loewi-effect' (i.e. the increase in discharge in the downstream preparation following stimulation of the upstream preparation) was depressed by hexamethonium, blocked by either mecamlamine or acetylcholinesterase and enhanced by physostigmine. The authors suggested that ACh was released during stimulation of the upstream carotid body and caused excitation of the downstream carotid body. Paintal (1969) has criticized these results on the basis that there would be insufficient ACh available in the carotid body to explain results in preparations which had not

been treated with anticholinesterases. Estimation of the ACh content of the cat carotid body by bioassay yields a value of about 20  $\mu\text{g/g}$  tissue (Eyzaguirre et al, 1965; Jones, 1975) which is comparable to the ACh content of the superior cervical ganglion. Nevertheless, since a cat carotid body weighs approximately 2 mg (Jones, 1975), the ACh content of the whole organ, based on these figures, is only of the order of 40 ng. The threshold concentration of ACh required to stimulate the cat carotid body *in vitro* is about 10 ng/ml (Eyzaguirre and Zapata, 1968b). Since the flow rates used in the 'Loewi-type' experiments were 0.6 - 0.1 ml/min and the period of stimulation was one minute, the upstream carotid body would have to release a minimum of 15 - 25 per cent of its entire store of ACh in order to achieve the threshold dose of ACh required to stimulate the downstream preparation. This proportion would in fact be much higher since some of the released ACh would be destroyed by cholinesterase enzymes. Such a situation seems unlikely. Recently Fidone, Weintraub and Stavinoha (1976) measured the ACh content of the cat carotid body by pyrolysis gas chromatography/mass fragmentography and reported values only a tenth of those found by bioassay. The authors suggested that this discrepancy may be due to the presence in the organ of another substance with ACh-like biological activity, e.g. carotidin (Christie, 1933). In any case it appears unlikely that the Loewi effect is due solely to ACh release from the upstream carotid body, although this may be a contributing factor. It is possible that stimulation of the upstream preparation increases its oxygen consumption thereby decreasing the oxygen tension of the superfusate and consequently reducing the oxygen supply to the downstream preparation. Such an idea has been



discussed by Eyzaguirre and Zapata (1968b) who considered it unlikely, and in any case this explanation does not allow for the effects of cholinergic blocking agents or of physostigmine. An alternative explanation may be that ACh modulates the release of a second substance, the identity of which is unknown, and it is this substance which is responsible for the Loewi-effect. If this were the case, one might expect drugs which affect the cholinergic system to modify responses in Loewi-type experiments and also only small amounts of ACh may be required to subserve such a role.

Metz (1969) measured the ACh content of the venous effluent from the dog carotid body and found that the concentration of ACh increased during hypoxia. In these experiments however, the venous blood was allowed to accumulate on a layer of muscle and consequently the source of the ACh in the samples could have been the muscle rather than the carotid body. Jones (1975) repeated Metz's experiments using cats instead of dogs but could not confirm his results.

#### Enzyme distribution and activity

Several workers have studied the distribution and activity of cholinesterase enzymes in the carotid body. Hollinshead and Sawyer (1945) found that the cat carotid body contained high levels of non-specific cholinesterase but only low levels of acetylcholinesterase. Since high levels of acetylcholinesterase normally occur in structures where ACh acts as a chemical transmitter, these authors considered it unlikely that ACh was a sensory transmitter in the carotid body. Koelle (1950, 1951) and Ross (1957) also found a predominance of non-specific cholinesterase in the cat carotid body, thus confirming the results of Hollinshead and Sawyer, and added that the cholinesterase

enzymes appeared to be localised in the type I (chief, glomus, parenchymal, epithelioid) cells and surrounding nerve fibres. Similar results have been reported for dog (Serafini-Fracassini and Frasson, 1966) and human (Pryse-Davies, Dawson and Westbury, 1964) carotid bodies. Fillenz and Woods (1966) found that in the rabbit carotid body, acetylcholinesterase was localised in type I cells while non-specific cholinesterase was located in nerve fibres and bundles around the cells. Korkala and Waris (1977) found acetylcholinesterase in type I cells in the rat carotid body.

Biscoe and Silver (1966) studied the effect of selective sectioning of the sinus nerve and the pre- and post-ganglionic sympathetic nerve supply on the distribution of cholinesterases in the cat carotid body. Their results suggested that both types of cholinesterase enzyme were associated with postganglionic sympathetic nerve fibres running from the superior cervical ganglion to the carotid body.

Although the level of acetylcholinesterase and its distribution is a useful indicator as to whether or not ACh is involved in transmission at efferent synapses, this may not be the case for afferent mechanisms. Lee (1968) suggested that a study of the choline acetylase activity would give a better indication as to whether or not cells are cholinergic. The level of choline acetylase in the carotid body appears to be very low however. Hebb (1968) found that choline acetylase activity in the cat carotid body was only 5 per cent of the activity found in the superior cervical ganglion and Jones (1975) has reported similarly low levels. Jones, however, pointed out that since there appears to be a predominance of non-specific cholinesterase in the carotid body and since this enzyme

hydrolyses ACh at a slower rate than does acetylcholinesterase, then relatively low levels of choline acetylase may be adequate to maintain stores of ACh in the carotid body.

### Pharmacological studies

#### 1. Anticholinesterases

Following the example of Schweitzer and Wright (1938) several groups of workers studied the effects of anticholinesterase drugs on the chemoreceptors. Heymans, Bouckaert and Pannier (1944) found that, in dogs, local application of physostigmine to the carotid body enhanced the chemoreceptor response to ACh but not to lobeline. This result was confirmed by Landgren, Liljestrand and Zotterman (1952) who used cats. Kaindl and Werner (1948) found that I.V. injection of low doses of physostigmine increased carotid chemoreceptor activity in the cat while higher doses abolished discharge. These results were confirmed by Landgren *et al* (1952) who further showed that injections of small doses of physostigmine, diisopropylfluorophosphate (DFP) or tetraethylpyrophosphate (TEPP) given close-arterially to the carotid body enhanced chemoreceptor discharge during normoxia, hypoxia or hyperoxia. This latter result was considered by the authors as evidence in favour of the idea that ACh acts as a sensory transmitter in the carotid body.

Landgren *et al* also confirmed the observation of Schweitzer and Wright (1938) that neostigmine has a stimulant effect on the chemoreceptors although they point out that neostigmine may have a direct cholinomimetic action as well as its better known anticholinesterase effects. This is an interesting point since Schweitzer and Wright did not observe chemoreceptor stimulation

following administration of physostigmine and their suggestion that ACh may act as a sensory transmitter was, as they themselves pointed out, based on the assumption that the stimulant effects of neostigmine were due to the anticholinesterase properties of the drug.

Liljestrand (1951, 1952) obtained further evidence in favour of the 'ACh hypothesis', i.e. the idea that ACh acts as a sensory transmitter in the carotid body. He found that local application of physostigmine to the carotid body of the cat leads to an increased respiratory response to hypoxia and also to hypercapnia.

Verbecke (1949a, 1949b), however, showed that in the dog both DFP and TEPP potentiate the response of the carotid chemoreceptors to ACh but not to KCN. Atanackovic (1950, 1951) found that, in the dog, tetramethoquinemethiodide, another anticholinesterase drug, potentiated the chemoreceptor response to ACh but not to nicotine, lobeline, sodium sulphide or KCN. A number of authors, also using dogs, found that the dimethylcarbamate derivative of hydroxyphenyl-trimethylammonium, a potent anticholinesterase drug, potentiated the chemoreceptor response to ACh (Mazzella and Migliaro, 1949; Atanackovic and Dalgaard-Mikkelsen, 1950) but not to sodium sulphide (Fernandez, 1949).

Douglas (1954) found, in contrast to Landgren *et al* (1952), that injection or infusion of TEPP into the carotid sinus region (species not specified) failed to produce any definite effect on the normal physiological response to hypoxia, although the response to injected ACh was greatly intensified. Heymans *et al* (1953) could find no increase of respiration in dogs following local application of physostigmine to the carotid body, unlike Liljestrand (1950, 1952) and Landgren *et al* (1952). Heymans *et al* did, however, observe a

slight potentiation of responses to lobeline and KCN after administration of physostigmine.

Daly (1954) has suggested that anticholinesterase drugs may increase chemoreceptor activity in the carotid body by an action on the superior cervical ganglion rather than directly on the carotid body itself. Anticholinesterase drugs might be expected to facilitate ganglionic neurotransmission and consequently increase sympathetic discharge to the carotid body. It is known that stimulation of the sympathetic supply to the carotid body leads to increased chemoreceptor activity in cats (Floyd and Neil, 1952). From the above results it may be seen that those workers who found that anticholinesterases enhance chemoreceptor activity and potentiate responses to chemoreceptor stimuli used cats as their experimental animal while those workers who found that this is not the case worked mainly with dogs. The discrepancy between these results might be explained in terms of a species difference if the sympathetic supply to the carotid body in dogs has less influence on chemoreceptor activity than the corresponding innervation in cats. Unfortunately, the influence of the sympathetic nerves on chemoreceptor activity in the dog does not seem to have been investigated.

Eyzaguirre and Koyano (1965b) studied the effects of physostigmine on chemoreceptor activity in the cat carotid body *in vitro* - a preparation which is free of the influence of the sympathetic nerves. They found that physostigmine caused an increase in chemoreceptor activity only if the superfusate contained CO<sub>2</sub>. Eyzaguirre and Zapata (1968a) obtained the same result using a similar preparation, but in addition they found that the chemo-

receptor response to acid was unaltered by physostigmine although the response to ACh was potentiated. It may be that the stimulant effects of physostigmine observed in these experiments were due to an action of this substance on the sensory nerve endings themselves rather than a consequence of blockade of cholinesterase enzymes. The CO<sub>2</sub> dependence of the response is difficult to explain in either case.

More recently, McQueen (1977) studied the effect of physostigmine on chemoreceptor activity in the cat carotid body *in situ*. He found that the chemoreceptor response to ACh was intensified following I.V. or close-arterial injection of physostigmine while responses to sodium cyanide (NaCN) or hypoxia were little affected. In these experiments the influence of sympathetic activity from the superior cervical ganglion on chemoreceptor discharge was removed by section of the ganglio-glomerular sympathetic nerves.

## 2. Nicotinic blocking agents

If ACh acted as a sensory transmitter in the carotid body in a manner analogous to cholinergic transmission in sympathetic ganglia then nicotinic blocking drugs would be expected to block the stimulation of afferent chemoreceptor activity produced by hypoxia or cyanide, as well as that produced by ACh or nicotinic stimulants.

Several reports have appeared concerning the effects of nicotinic blocking drugs on the response of the carotid chemoreceptors to various stimuli. Moe, Capo and Peralta (1948) found that in dogs continuous I.V. infusion of tetraethylammonium (TEA) blocked the chemoreceptor response to ACh, nicotine and lobeline



but not that to hypoxia or cyanide. Other workers obtained similar results to Moe *et al* using hexamethonium in anaesthetized cats (Douglas, 1952) and TEA, hexamethonium and pendiomide in anaesthetized cats and dogs (Dontas and Nickerson, 1956). Byck (1961) found that in dogs hexamethonium blocked the chemoreceptor response to nicotine but not to cyanide. Boelaert (1948) reported that TEA had no effect on the sensitivity of the carotid chemoreceptors in dogs but does not mention the stimulus used in his experiments. Heymans, Delaunois, Martini and Janssen (1953) found that in dogs, local application or I.V. injection of TEA, hexamethonium, methantheline or pendiomide did not block the chemoreceptor stimulation produced by ACh, lobeline or cyanide although, as Byck (1961) points out, the results presented in their paper show a clear reduction in the response to lobeline after administration of hexamethonium.

These observations presented a serious objection to the ACh hypothesis. In contrast to the above results, however, Landgren *et al* (1952) found that in cats, local injections of TEA or decamethonium abolished the response of the carotid chemoreceptors to ACh or lobeline and greatly reduced responses to hypoxia. Gollwitzer-Meier and Witzleb (1953) found that local injections of decamethonium, at a dose less than that required to produce respiratory paralysis, had no effect on afferent chemoreceptor discharge but pentamethonium inhibited the chemoreceptor response to ACh, lobeline and hypoxia. More recently, Joels and Neil (1963) studied the effects of hexamethonium on chemoreceptor activity in the vascularly isolated carotid body in cats and found, in contrast to Douglas (1952), that this ganglion blocking drug not only reduced the chemoreceptor response to ACh but also that to hypoxia, 2,4-dinitrophenol

or cyanide. Joels and Neil, however, were not of the opinion that ACh acts as a sensory transmitter in the carotid body.

It is worth noting that although Landgren *et al* (1952) observed a reduction in the chemoreceptor response to hypoxia following administration of TEA or decamethonium, the dose of blocking drug required to produce this effect was much greater than the dose required to block responses to ACh or lobeline. Gollwitzer-Meier and Witzleb (1953) also gave high doses of blocking drug close-arterially. Joels and Neil (1963) do not mention the dose of hexamethonium used in their experiments. In order to explain this difference in sensitivity, Landgren *et al* (1952) suggested, on the basis of the morphological studies of De Castro (1951) that the functional chemoreceptor synapses are intracellular, i.e. the sensory nerve endings form end plates within the cytoplasm of the glomus cells, and that these sites are inaccessible to nicotinic blocking drugs. Moe *et al* (1948) made a similar suggestion. This implies, however, that both nicotinic blocking drugs and cholinergic stimulants act at an extrasynaptic site. In any case, it is now known that nerve endings in the carotid body do not invade the cytoplasm of glomus cells but terminate extracellularly (Biscoe, 1971).

ACh can stimulate a variety of sensory receptors and this effect may be blocked by cholinergic blocking agents without affecting the response of these receptors to normal physiological stimulation (for references see Diamond, 1955). Douglas (1954) drew attention to the similarities between stimulation of the carotid chemoreceptors by ACh and the action of this substance on other sensory receptors and suggested that this may be a non-specific effect of ACh which is "altogether independent of normal transmission mechanisms" in these structures.



Eyzaguirre and Koyano (1965b) reinvestigated the effects of nicotinic blocking agents on chemoreceptor activity in the cat carotid body *in vitro*. They found that hexamethonium, D-TC and gallamine stimulated chemoreceptor activity in small concentrations and induced depression of sensory discharge in higher concentrations. Responses to NaCN and anoxia were either unaffected or slightly reduced by these drugs. The authors did not consider their results inconsistent with the ACh hypothesis but suggested that ACh may mediate chemoreceptor impulses during mild chemoreceptor stimulation while more intense stimulation, such as that produced by NaCN or anoxia, may involve a separate mechanism.

Eyzaguirre and Zapata (1968a) found that the response of the cat carotid chemoreceptors *in vitro* to acid or to ACh was reduced by hexamethonium, mecamlamine, or dihydro- $\beta$ -erythroidine (DHE). The response to anoxia (stoppage of flow) was depressed only by mecamlamine and DHE. In a second paper (Eyzaguirre and Zapata, 1968b) it was reported that although the chemoreceptor response to ACh was blocked by mecamlamine, hexamethonium and D-TC this blockade could be overcome, at least partially, by higher doses of ACh. Since the concentration of ACh in the synapse during strong chemoreceptor stimulation is likely to be high, they argued that this might overcome the effect of the blocking agents. They further argued that chemoreceptor synapses, which they considered as a sensory nerve end apposed to a type I cell, might be fairly inaccessible to quaternary cholinergic blocking agents such as hexamethonium due to the presence of sustentacular (type II) cells enveloping the type I cells. Mecamlamine being a simple tertiary amine, more readily penetrates membranes and is therefore more effective.

Sampson (1971) found that I.V. injection of mecamlamine in the anaesthetized cat blocked the carotid chemoreceptor response to ACh but had no effect on responses to NaCN, 0.1 N HCl, or hypoxia. Similar results were obtained by Nishi and Eyzaguirre (1971) who <sup>given</sup> found that injections of small doses of mecamlamine <sub>^</sub>close-arterially to the cat carotid body *in situ* blocked the chemoreceptor response to ACh but not that to NaCN. The latter authors, however, also found that larger doses of mecamlamine blocked the response to both ACh and NaCN. They showed that the effects of large doses of mecamlamine were not due to a local anaesthetic action and argued that the lack of effectiveness of small doses of the drug in blocking the response to NaCN was due to the high concentration of ACh released into the synaptic cleft overcoming the blocking effects. These authors also studied the effects of hexamethonium on the response of the carotid chemoreceptors to NaCN and ACh but could find no consistent effect on responses to either substance.

Eyzaguirre and Nishi (1974) studied the effects of mecamlamine on the 'mass receptor potential' of the cat carotid chemoreceptors *in vivo*. The mass receptor potential is taken as a cumulative measure of the depolarization of sensory nerve endings in the carotid body. They found that close-arterial administration of high doses of mecamlamine blocked the 'receptor depolarization' produced by either ACh or NaCN.

Thus a conflict of evidence has emerged which may be interpreted in favour of the ACh hypothesis or against it. In the opinion of McQueen (1977) this conflict has arisen as a result of subjective interpretation, by various authors, of qualitative pharmacological data, in terms of whether the authors did or did not support the ACh

hypothesis. McQueen (1977) quantified the effects of various blocking agents by calculating the ratio of the dose of a stimulant required to produce a given response before administration of a blocking agent to the dose required to produce the same response after. When sufficient data were available from the present experiments, this method was used to quantify results presented in this thesis (see Section II). Using this quantitative method, McQueen showed that the chemoreceptor response to ACh was completely blocked by mecamylamine while responses to hypoxia or NaCN were unaffected even after high doses of mecamylamine given close-arterially. He also found that  $\alpha$ -bungarotoxin, a neuromuscular blocking agent, caused a slight reduction in responses to both ACh and NaCN.

Data from experiments with nicotinic blocking agents do not support the acetylcholine hypothesis. While it may be argued that high synaptic concentrations of ACh can overcome the effects of blocking agents, this does not occur at other cholinergic synapses such as are found in sympathetic ganglia or at the neuromuscular junction. The argument that the sensory synapse in the carotid body is inaccessible to cholinergic blockers does not apply to mecamylamine since this substance passes easily through membranes. In those experiments where mecamylamine was found to be an effective blocker of chemosensory activity, very high doses of the drug were required and under these conditions, non-specific actions may have been responsible for the observed effects.

### 3. Atropine

Stimulation of carotid chemoreceptors in cats and dogs by ACh has been shown to be mediated by nicotinic receptors (see above).

Atropine is a drug which is useful mainly for its ability to block muscarinic cholinergic effects although it also has nicotinic blocking properties at high doses (Ambache, 1955). Nevertheless, atropine has been used by several groups of workers to investigate whether or not ACh acts as a sensory transmitter in the carotid body.

The earliest reports available in the literature showed that large I.V. doses of atropine had no significant effect on the respiratory response to ACh (Schweitzer and Wright, 1938; Euler, Liljestrand and Zotterman, 1939; Schmidt and Comroe, 1940). Liljestrand (1952), however, found that local application of a one per cent solution of atropine sulphate (approximately  $1.4 \times 10^{-3}$  mol./l) to the carotid body of the cat decreased the response of the carotid chemoreceptors to ACh, lobeline, hypoxia or hypercapnia. Anichkov (1953) similarly found that atropine reduced the chemoreceptor response to ACh. Heymans *et al* (1953), however, showed that these results could be adequately explained by the local anaesthetic action of atropine.

Eyzaguirre and Koyano (1965b) found that atropine reduced spontaneous chemoreceptor activity in the cat carotid body *in vitro* but had little effect on the response to anoxia or NaCN. Eyzaguirre and Zapata (1968a) showed that atropine reduced the chemoreceptor response to acid *in vitro*. The dose of atropine used was of the order of  $10^{-5}$  -  $10^{-4}$  mol./l. In a second paper in the same series (Eyzaguirre and Zapata, 1968b), they showed that doses of atropine of the order of  $10^{-6}$  -  $10^{-5}$  mol./l reduced the response of the carotid chemoreceptors to ACh. At these doses, however, it is quite possible that the observed effects were due to the nicotinic blocking action of atropine or to local anaesthetic effects. Nishi and Eyzaguirre

(1970, 1971), however, showed that atropine blocked the response of cat carotid chemoreceptors *in vivo* to NaCN and ACh and that this was not due to a local anaesthetic action of the drug. Eyzaguirre and Nishi (1974) showed that atropine blocked 'receptor depolarization' induced by ACh or NaCN. In a quantitative study of the effects of blocking drugs on chemoreceptor responses, McQueen (1977) found that atropine caused a slight reduction in responses to both NaCN and ACh.

Since stimulation of the chemoreceptors by ACh appears to be mediated solely by nicotinic receptors (McQueen, 1974), presumably blockade of the stimulant effects of ACh by atropine is due to a nicotinic blocking action. In any case, it is not clear from the above studies whether or not atropine, in doses below those expected to produce local anaesthetic effects, has any effect on normal chemoreceptor transmission mechanisms.

#### 4. Drugs acting presynaptically

Another pharmacological approach to the study of possible cholinergic mechanisms in the carotid body has involved the use of drugs which affect the synthesis or release of ACh. Hemicholinium-3 (HC-3) is a drug which is thought to act by preventing the uptake of choline into cholinergic neurons and thereby limiting the synthesis of ACh. Eyzaguirre and Zapata (1968a) found that the response of the cat carotid chemoreceptors *in vitro* to NaCN was reduced after prolonged exposure to HC-3 while the response to ACh was unaffected. They also showed that the NaCN response could be partially restored by washing the preparation with Locke solution containing choline. Similar results were obtained using HC-3



*in vivo* (Nishi and Eyzaguirre, 1971; Eyzaguirre and Nishi, 1974). McQueen (1977) also studied the effects of HC-3 *in vivo*. He found that although responses to NaCN were less intense following administration of HC-3, the duration of the response was increased, the net effect being a potentiation. Responses to ACh were little affected.

$\beta$ -Bungarotoxin is a substance reputed to act by preventing the release of ACh at the neuromuscular junction (Chang, Chen and Lee, 1973). McQueen (1977) found that this drug had very little effect on the response of cat carotid chemoreceptors to NaCN but slightly depressed responses to ACh. This result does not support the cholinergic hypothesis although it is possible, if ACh is a sensory transmitter in the carotid body, that the mechanism for ACh release in the carotid body differs from that at the neuromuscular junction.

The results discussed above from experiments with HC-3 might be interpreted in favour of the ACh hypothesis although, as was the case for experiments with postsynaptic blockers and anticholinesterases, they are equivocal and far from conclusive.

The ACh hypothesis is clearly a controversial issue. Although several studies, employing various techniques, have been made in an attempt to reconcile the controversy it is still uncertain whether or not ACh is a sensory transmitter in the carotid body. The weight of evidence, however, seems to be against the supposition. In addition to its proposed role as a sensory transmitter, ACh has also been suggested as a possible transmitter in an inhibitory efferent pathway which runs in the sinus nerve (Neil and O'Regan, 1971). This proposal, however, also requires ratification.

## CATECHOLAMINES

Electron-microscopic studies of the fine structure of the carotid body have shown that the type I cells contain numerous dense-cored vesicles (DCVs) throughout their cytoplasm (Lever and Boyd, 1957; Garner and Duncan, 1958; Hoffman and Birrel, 1958). It has been suggested that these DCVs represent a store of phenolic amines which might be involved in the initiation of chemoreceptor impulses (Lever, Lewis and Boyd, 1959).

Evidence from fluorescence microscopic and autoradiographic studies of catecholamines in the carotid body strongly supports the idea that these substances are stored in DCVs in type I cells (see Biscoe, 1971). Results from quantitative studies of the catecholamine content of the carotid body have been equivocal (see Table 1.1). Most authors agree that DA is the most abundant catecholamine in the carotid body with about 25 - 75 per cent as much noradrenaline (NA) and smaller amounts of adrenaline (ADR) also present. Recently, however, Mills, Smith, Slotkin and Breese (1978) found four to five times as much NA as DA in the cat carotid body and have contested the idea that DA is the most abundant of these two catecholamines in the carotid body of this species.

It is possible that the NA content of the carotid body is stored in sympathetic nerve endings rather than in type I cells. Chronic sympathectomy (3 - 7 days), however, has little effect on the amine content (Zapata, Hess, Bliss and Eyzaguirre, 1969) or on dopamine- $\beta$ -hydroxylase activity (Morgado, Lladós and Zapata, 1976) of the cat carotid body, although in this species the carotid body receives a post-ganglionic sympathetic innervation which is thought to be noradrenergic (O'Regan, 1977). Presumably, in the cat, the

TABLE 1.1: Summary of results from quantitative studies of the catecholamine content of the carotid body (c.b.)

Author	Date	Species	DA	NA	ADR	Units
Chiocchio <i>et al</i>	1966	cat	134 ± 24	81 ± 9	15 ± 3	ng/c.b.
Chiocchio <i>et al</i>	1967	cat	4.4 ± 0.4	2.4 ± 0.4	0.4 ± 0.1	µg/g
Zapata <i>et al</i>	1969	cat	204	98	24	ng/c.b.
Chiocchio <i>et al</i>	1971	cat	260	31 ± 51	not reported	ng/c.b.
Mills <i>et al</i>	1975	cat	not reported	82 ± 8*		ng/c.b.
Escheverria <i>et al</i>	1977	cat	56.5	40.9	3.4	µg/g
Mills <i>et al</i>	1978	cat	34 ± 2	162 ± 16	not reported	ng/c.b.
Hellström <i>et al</i>	1975	rat	4.4 ± 1.5	2.9 ± 0.2	not reported	ng/pair c.b.
Hellström <i>et al</i>	1976	rat	4.6 ± 0.3	1.2 ± 0.04	not reported	ng/pair c.b.
Hellström	1977	rat	4.4 ± 1.2	1.2 ± 0.04	not reported	ng/pair c.b.
Hanbauer <i>et al</i>	1978	rat	2.6 ± 0.2	0.9 ± 0.1	not reported	ng/pair c.b.
Dearnaley <i>et al</i>	1968	rabbit	21.1 ± 3.6	not reported	not reported	ng/c.b.
Lishajko	1970	human**	8.2	2.0	3.2	µg/g

\*Combined value for NA and ADR

\*\*Carotid body tumour



contribution of dopamine- $\beta$ -hydroxylase activity and NA content of sympathetic nerve fibres to that of the whole organ is small. In the rat, chronic sympathectomy (5-7 days) reduces the NA content of the carotid body by about 50 per cent (Hanbauer and Hellström, 1978).

It seems that the NA content of the sympathetic nerve endings does not account for all the NA stored in the carotid body. It is therefore possible that both NA and DA are stored in type I cells although there may be species differences in the ratio of NA to DA stored in these structures.

It is not clear whether NA and DA are stored in the same DCV, in separate DCVs in the same cell, or in separate DCVs in separate cells. Some authors have been able to recognise at least two varieties of type I cell on the basis of the electron density of their cytoplasm (Lever, Lewis and Boyd, 1959; Hoglund, 1967; Grimley and Glenner, 1968; Chen, Yates and Duncan, 1969; Morita, Chiocchio and Tramezzani, 1969; Abbot, Daly and Howe, 1972) while others have made a similar distinction based on the size and electron density of DCVs in different cells (Kobayashi, 1968, 1975; Morita *et al*, 1969; McDonald and Mitchell, 1975; Hellström, 1975). It may be that different amines are stored in separate DCVs, and possibly separate cells, but while the above results are suggestive, there is no conclusive evidence to support such a notion.

The finding that catecholamines are stored in the carotid body raises three important questions:

- a) Are these substances involved in the mechanism of generation of afferent chemoreceptor activity?
- b) Are these substances released from the carotid body and if so, under what conditions?

- c) Does the carotid body have a second function which is either related to or independent of its role in arterial chemoreception, e.g. as an organ of internal secretion?

The notion that the carotid body acts as an organ of internal secretion has received limited support (see Karnauchow, 1965; Chen *et al*, 1969; Pearse, 1969; Kobayashi, 1971, 1975) but is not yet generally accepted. The conditions under which catecholamines are released in the carotid body and the possible significance of these substances in the chemoreceptive mechanism are, however, subjects which have received a great deal of attention, especially in recent years, and are of particular relevance to the present discussion.

#### Pharmacological effects

Several studies have been made of the effects of catecholamines on chemoreceptor activity in the carotid body. Witzleb (1953) found that local application of either ADR or NA to the carotid body in cats caused no detectable change in chemoreceptor activity. Kuznetsov and Belen'kii (see Anichkov and Belen'kii, 1963), however, showed that ADR, perfused into the isolated carotid artery, induced mild respiratory excitation in decerebrate cats. Similar results were obtained by Lee (see Torrance, 1968) who attributed this effect to a reduction in carotid body blood flow secondary to vasoconstriction. Joels and Neil (1968) found that ADR and NA, but not DA, caused an increase in chemoreceptor activity in the perfused cat carotid body which they also attributed to vascular effects. Studies of the effects of NA and ADR on chemoreceptor activity in the superfused cat carotid body *in vitro*, a preparation in which vascular effects are precluded, showed that these substances do not stimulate chemo-

receptor activity but, if anything, cause a depression of activity (Eyzaguirre and Koyano, 1965b).

Experiments in dogs yielded quite different results. Byck (1957) found that in this species a variety of sympathomimetic amines, including NA and DA, caused reflex hyperpnoea by an action on carotid chemoreceptors. Jacobs and Comroe (1968) confirmed Byck's findings and showed that on a molar basis, DA was three times more potent than NA as a chemoreceptor stimulant. The short latency of the response suggested to the authors that this was likely to be due to a direct action of catecholamines on chemoreceptor elements in the carotid body rather than a response to reduced blood flow brought about by vascular changes. They also found that chemoreceptor stimulation produced by NaCN or by catecholamines was reduced by phentolamine, an  $\alpha$ -adrenergic antagonist, although this effect was not consistent. Propranolol, a  $\beta$ -adrenergic antagonist, had no apparent effect on the response. Heymans, De Schaepdryver and De Vleeschhouwer (1968) found that neither  $\alpha$ -blockade with phenoxybenzamine (PBA) nor  $\beta$ -blockade with propranolol had any effect on chemoreceptor responses to NaCN or hypoxia in dogs. The latter authors, however, did not study the effect of these blocking drugs on the chemoreceptor response to catecholamines.

As well as their work on dogs, Jacobs and Comroe (1968) reported preliminary results from experiments on cats which suggested that DA did not stimulate carotid chemoreceptor activity in this species. This observation was confirmed in a subsequent study (Black, Comroe and Jacobs, 1972) in which it was shown that DA depressed chemoreceptor activity in cats. NA was found to cause a slight depression of chemoreceptor activity in some experiments but

had no consistent effect in this species. Similar results were reported by Sampson (1972) using anaesthetized cats, although this author obtained more consistent inhibitory responses to NA than did Black *et al* (1972), and in addition found that ADR inhibited chemoreceptor activity. It was also found that inhibitory responses to NA and ADR were followed by a period of increased activity, an effect which the author attributed to vasoconstriction.

The above evidence indicates that the effects of catecholamines on carotid chemoreceptor activity are species dependent. DA is found to depress chemoreceptor activity in the cat *in vivo* while NA and ADR cause a stimulation of activity which is probably due to vasoconstriction and may be preceded by a brief inhibitory effect. Both DA and NA stimulate chemoreceptor activity in the dog by a mechanism as yet unknown.

Results from studies of the effects of catecholamines on carotid chemoreceptor activity *in vitro* have been equivocal. In contrast to the results of Eyzaguirre and Koyano (1965b) discussed above, Biscoe (1965) found that NA, ADR and the  $\beta$ -adrenergic agonist isoprenaline all stimulated chemoreceptor activity in the superfused cat carotid body *in vitro* and that the chemoreceptor response to these substances as well as that to ACh or hypoxia was blocked by the  $\beta$ -adrenergic antagonists nethalide and dichloroisoprenaline (DCI). Zapata, Hess, Bliss and Eyzaguirre (1969), however, using a similar preparation to Biscoe, found that catecholamines neither excited nor depressed chemoreceptor activity and that the inhibitory effects of DCI were due to the local anaesthetic properties of this drug rather than its  $\beta$ -receptor blocking action.

Zapata (1975) reinvestigated the effects of DA and NA on chemoreceptor activity *in vitro*. In agreement with his earlier study (Zapata, Hess, Bliss and Eyzaguirre, 1969), the author reported that NA did not affect chemoreceptor activity. In contrast to previous results, DA was found to cause a transient depression of chemoreceptor activity. Following repeated injections of DA administered at short intervals, the inhibitory response was changed to a biphasic response, i.e. an initial inhibitory effect followed by stimulation of activity. Successive injections of DA ultimately resulted in a pure excitatory response. DL-Dopa, a precursor to DA in the synthetic pathway, was also found to produce excitatory effects. The inhibitory effects of DA but not its excitatory effects were blocked by spiroperidol, a DA receptor antagonist, or by dibenamine, an  $\alpha$ -adrenergic antagonist.

It has also been found that DA-induced inhibition of chemoreceptor activity in the cat carotid body *in situ* is blocked by  $\alpha$ -adrenergic blocking agents (Sampson, 1972, 1975; Sampson, Aminoff, Jaffe and Vidruk, 1976b; Mitchell and McDonald, 1975) although some workers have found that this is not the case (Nishi, 1977; Zapata, 1977; Lladós and Zapata, 1978b). There is, however, general agreement in the literature that DA receptor blocking agents of the neuroleptic type can block the inhibitory effects of exogenously administered DA on chemoreceptor activity in the cat carotid body (Zapata, 1975, 1977; Nishi, 1977; Lladós and Zapata, 1978a).

There is evidence to suggest that DA may influence responses to chemoreceptor stimuli. Zapata (1975) has shown in the cat carotid body *in vitro* that in preparations in which DA evokes inhibition of chemoreceptor activity, the inhibitory effect of DA



may partially or totally counteract the chemoreceptor stimulating effects of simultaneously applied ACh or NaCN, while in preparations in which DA has excitatory effects the stimulation produced by simultaneously applied ACh or NaCN is additive. Nishi (1977) has shown in the cat carotid body *in situ* that the inhibitory effects of DA may counteract the stimulant effects of simultaneously applied NaCN or of hypoxia.

Attempts have been made to study the importance of endogenous catecholamines in the carotid body by observing the effects of pharmacological depletion of catecholamines, using reserpine or 6-hydroxydopamine (6-OHDA), on chemoreceptor activity. It has been variously reported that prolonged exposure to reserpine in cats reduces chemoreceptor responses to ACh (Koppanyi and Cowan, 1962) or to hypoxia (Cowan and Greene, 1965), completely abolishes chemoreceptor activity (Comroe, 1964) or has no effect on chemoreceptor activity or responsiveness (Zapata, Hess, Bliss and Eyzaguirre, 1969; Nishi, 1975). Attempts to deplete the carotid body of its catecholamine content with 6-OHDA have met with varying degrees of success. It has been found that chronic treatment with 6-OHDA causes a reduction in catecholamine fluorescence in the rat carotid body (Lassman and Bock, 1972; Hess, 1975) but other workers have found that neither catecholamine fluorescence (Hansen and Ord, 1978) nor catecholamine content (Hellstrom, 1977) are affected by chronic exposure to 6-OHDA in this species. Zuazo and Zapata (1978) found that carotid chemoreceptor responses to NaCN and hypoxia in the cat were unchanged up to seven hours after intracarotid injections of 6-OHDA but made no estimate of the degree of catecholamine depletion, if any, produced by 6-OHDA in these experiments. Murphy and O'Regan (1977)

found that the chemoreceptor response to mild hypoxia in cats was slightly potentiated 10 - 12 min after administration of 6-OHDA but did not investigate the effects of chronic exposure to the drug and, like Zuazo and Zapata, made no estimate of the degree of catecholamine depletion produced by 6-OHDA in their experiments.

Clearly, exogenously applied catecholamines can influence chemoreceptor activity although the effects of catecholamines, especially DA, are complex. Results from studies of the effects of pharmacological depletion of catecholamines on chemoreceptor activity in the carotid body allow no clear conclusion as to the role, if any, of catecholamines in the mechanism of generation of afferent chemoreceptor activity. If, however, catecholamines do play a role as chemical transmitters in the carotid body then presumably they must be released from type I cells in order to have any effect on chemoreceptor activity in the normally functioning carotid body. It is important therefore to ascertain the conditions under which catecholamines are released from type I cells.

#### Release of catecholamines

Several studies of the effects of hypoxia on the number and density of DCVs in the type I cells of the carotid body have appeared in the literature. Results from such studies have, however, been inconclusive. It has been reported that the number of DCVs in type I cells following hypoxia is either unchanged (Zapata, Hess, Bliss and Eyzaguirre, 1969) or increased (Al-Lami and Murray, 1968) in the cat, unchanged in the hamster (Chen *et al*, 1969), increased in the rabbit (Moller, Mollgard and Sorensen, 1974) and reduced in the rat (Hoffman and Birrel, 1958; Blumcke, Rode and Niedorf, 1967). Results



such as these are difficult to interpret since the number of DCVs may not reflect the amine content of the cell and a reduced number of DCVs may be produced by pathological changes rather than a specific effect of hypoxia.

Zapata, Hess, Bliss and Eyzaguirre (1969) measured the amounts of DA, NA and ADR in the cat carotid body before and after chemoreceptor stimulation by hypoxia both *in vivo* and *in vitro* (control values are given in Table 1.1) but found no obvious difference in the amounts of these substances under the different experimental conditions. Mills and Slotkin (1975), however, found that the total NA plus ADR content of the cat carotid body (control values are given in Table 1.1) was reduced following hypoxia and that the extent of the reduction was dependent on the magnitude and the duration of the hypoxic stimulus. The effect of hypoxia on DA levels was not measured in the latter study.

Hellström, Hanbauer and Costa (1976) measured catecholamine levels in the rat carotid body under a variety of different experimental conditions using a highly sensitive gas chromatographic-mass spectrometric assay method. They found that there was a significant reduction in the DA content but no change in the NA content of carotid bodies removed from rats exposed to hypoxia. This result was obtained whether or not the carotid sinus nerve was intact. They also found that DA depletion by hypoxia was partially relieved by administration of atropine and that the muscarinic agonist methacholine, like hypoxia, caused a preferential reduction in DA levels. These observations suggested to the authors that DA is released from type I cells during hypoxia and that DA release may be triggered by ACh acting on muscarinic receptors located on the membrane of the

type I cells. In this scheme ACh would be released from "intrinsic cholinergic neurons" or from the type I cells themselves.

The finding that the DA content of the rat carotid body, but not its NA content, is reduced by hypoxia has been confirmed by Hanbauer and Hellström (1978) who further showed that this effect is not due to a change in DA turnover and is best explained by an increased release of DA. There is also evidence to suggest that DA is released during chemoreceptor stimulation in the rabbit carotid body (Gonzalez and Fidone, 1977).

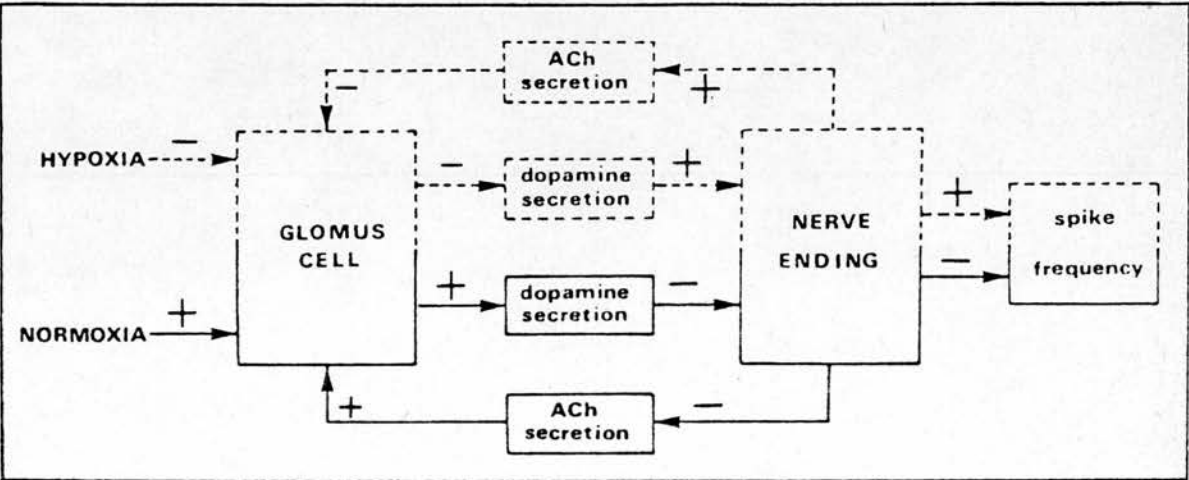
Although the NA content of the rat carotid body is not reduced by hypoxia, it has been found that chronic section of the sinus nerve causes an increase in the NA content of the carotid body in this species (Hanbauer and Hellström, 1978). Other evidence also suggests that the sinus nerve may have an influence on catecholamine levels in the carotid body. Mills and Slotkin (1975) found that cutting the sinus nerve attenuated the depletion of NA and ADR produced in the cat carotid body by hypoxia (see above). It has been found that electrical stimulation of the sinus nerve causes depletion of DCVs in type I cells in the hamster carotid body and that this effect is blocked by atropine (Yates, Chen and Duncan, 1970). Few conclusions can be drawn from this latter result since the dose of atropine used in these experiments was 200 mg/kg, and at this dose pronounced anaesthetic effects would be expected. Sampson, Nicolayson and Jaffe (1975) reported that electrical stimulation of the peripheral cut end of the sinus nerve resulted in an increase in the intensity of formaldehyde-induced fluorescence intensity in type I cells of the carotid body of normal cats and a reduction in the fluorescence intensity in type I cells in cats pretreated with

MK-486, a dopamine- $\beta$ -hydroxylase inhibitor. The authors interpreted these results as evidence suggesting that efferent discharge in the sinus nerve stimulates the synthesis and release of catecholamines.

Role of catecholamines - hypotheses

A number of theories have been advanced in which DA is considered to play an important role in the mechanism of chemoreception. Osborne and Butler (1975) have proposed a model which suggests that DA is released from type I cells in the carotid body and acts to inhibit activity in otherwise spontaneously active sensory nerve endings- when the chemoreceptors are stimulated DA secretion is reduced and consequently afferent nerve activity increases. This hypothesis also suggests that a chemical transmitter, possibly ACh, is released from the sensory nerve endings and acts on type I cells to further reduce DA secretion thus constituting a positive feedback loop (see Figure 1.1).

FIGURE 1.1: Schematic flow diagram of carotid body chemoreceptor during hypoxia (upper, dotted lines) and normoxia (lower, solid lines) according to the hypothesis of Osborne and Butler (1975) (Taken from original paper - Nature 254 pp. 701-703)



The central feature of Osborne and Butler's hypothesis is that increased chemosensory activity arises as a result of disinhibition of spontaneously active nerve endings rather than excitation of otherwise quiescent nerve endings. Krammer (1978) introduced an hypothesis based on a similar idea. This author envisages a model in which there are two varieties of type I cell - DA-containing type IA cells and NA-containing type IB cells. It is suggested that DA is continually released from type IA cells and acts on otherwise active sensory nerve endings to suppress activity. When the chemoreceptors are stimulated, NA is released from type IB cells to decrease DA secretion and consequently cause disinhibition of afferent nerve activity. Both Osborne and Butler's and Krammer's hypotheses suggest that DA release decreases during chemoreceptor stimulation. Evidence available at present, however, suggests that the DA content of the carotid body is either unchanged or decreased by hypoxia (see above) which does not support such an idea.

McDonald and Mitchell (1975) have proposed an alternative hypothesis. According to them, the sensory nerve ending is itself the chemoreceptor while type I cells are dopaminergic interneurons in a local control mechanism. It is suggested that afferent nerve endings are connected with type I cells by reciprocal synapses, i.e. nerve ending and type I cell are both presynaptic and postsynaptic to each other. Sensory nerve endings, when stimulated, release a chemical transmitter which acts to promote DA release from type I cells. DA, released in this way, then acts on the sensory nerve ending to inhibit sensory activity thus forming a negative feedback control system. According to this hypothesis, DA release increases during chemoreceptor stimulation. Zapata (1975) has also suggested that endogenous DA may modulate afferent chemoreceptor activity.



The hypotheses discussed above suggest possible roles for catecholamines, especially DA, in the mechanism of generation of afferent chemoreceptor activity. The multiplicity of these hypotheses is in some ways a measure of the difficulty involved in establishing a role for catecholamines in the mechanism of carotid chemoreception. The mammalian carotid body contains sufficient amounts of catecholamines for them to subserve a role as chemical transmitters in this organ, but if such a role exists it remains unknown at present.

#### 5-HYDROXYTRYPTAMINE

In a histological study of human carotid body tumour, Costero and Barosso-Miguel (1961) identified granule-containing cells, distinct from type I cells, which they suggested might contain 5-HT. No evidence for the presence of 5-HT in human carotid body tumours was found in subsequent studies by fluorescence histochemistry (Niemi and Ojala, 1964) or paper chromatography (Pryse-Davies, Dawson and Westbury, 1964). Hamberger, Ritzen and Wersall (1966), however, identified 5-HT containing cells in the normal human carotid body by fluorescence microspectrophotometry.

Quantitative estimations of 5-HT content have been made for cat and rat carotid bodies - the values obtained are shown in Table 1.2. Values obtained for DA content in these studies are also included in the table for comparison.

There is evidence to suggest that 5-HT, like DA or NA, is stored in DCVs in type I cells in the carotid body in cats (Chiocchio *et al*, 1967) and hamsters (Chen *et al*, 1969). Gronblad and Korkala (1977) have suggested, however, that 5-HT is stored in interlobular mast cells in the rat carotid body. Chiocchio *et al* (1967)

postulated that 5-HT might act as a chemical transmitter in the carotid body.

TABLE 1.2: Summary of results from quantitative studies of 5-HT content in the carotid body (c.b.). Values for DA content obtained in the same experiment are included for comparison - see also Table 1.1

Author	Date	Species	5-HT	DA	Units
Chiocchio <i>et al</i>	1967	cat	$6.9 \pm 0.3$	$4.4 \pm 0.4$	$\mu\text{g/g}$
Chiocchio <i>et al</i>	1971	cat	10*	260	$\text{ng/c.b.}$
Hellström	1977	rat	$7.5 \pm 1.4$	$28.9 \pm 7.9$	$\text{pmol/pair c.b.}$

\* estimated value assuming cat c.b. weighs approximately 2 mg (Jones, 1975).

#### Pharmacological studies

I.V. injection of 5-HT stimulates respiration in the dog (Page, 1952). This phenomenon has been attributed to an action of 5-HT on carotid chemoreceptors (Douglas and Toh, 1952; Comroe and Mortimer, 1964), an action of 5-HT on the C.N.S. (Heymans and Heuval-Heymans, 1953), or to stimulation of cardio-pulmonary reflexes (Schneider and Yonkman, 1954). Schneider and Yonkman (1954) found that the effects of 5-HT on respiration were species-dependent, the normal effect in dogs and rabbits being excitation of respiration while in cats 5-HT has a biphasic effect, i.e. an initial inhibition followed by excitation of respiration. Ginzell and Kattegoda (1954) also found that the respiratory response to I.V. injection of 5-HT in the cat consisted of an initial inhibitory and a secondary excitatory phase and showed that the secondary excitation was abolished by sinus nerve section.



McCubbin, Green, Salmoiraghi and Page (1956) recorded afferent chemoreceptor activity in the sinus nerve of the dog. They found that intracarotid injection of 5-HT caused a marked stimulation of chemoreceptor activity which suggests that the respiratory stimulant effects of 5-HT in this species are due, at least in part, to stimulation of carotid chemoreceptors. This view is supported by the results of Black *et al* (1972) who found that 5-HT consistently produced immediate hyperpnoea in dogs when the carotid sinus nerve was intact but not when it was cut.

Studies of the effects of 5-HT on afferent chemoreceptor activity in cats have yielded quite different results. Eyzaguirre and Koyano (1965b) found that 5-HT normally caused a slight depression of chemoreceptor activity in the cat carotid body *in vitro* although in one experiment 5-HT was found to stimulate activity. Black *et al* (1972) found that the effects of 5-HT on chemoreceptor activity in the cat carotid body *in situ* were varied, stimulation of activity occurring in some experiments while in others 5-HT caused depression of activity, or lacked effect.

More recently, Nishi (1975) reinvestigated the effects of 5-HT and also studied the effects of some antagonists of 5-HT on chemoreceptor activity in the cat carotid body *in situ*. He found that 5-HT caused a brief intense stimulation of chemoreceptor activity followed by a depression or blockade of activity lasting several seconds. Repeated injections of 5-HT at short intervals led to progressively smaller responses, i.e. the preparation became desensitized to the effects of 5-HT. This might be the reason why previous workers found that chemoreceptor responses to 5-HT were inconsistent in this species. Nishi also found that the effects of

5-HT on carotid sinus baroreceptor activity were qualitatively similar to its effects on chemoreceptor activity which led him to suggest that these effects were due to a non-specific action of 5-HT on sensory nerve endings and that 5-HT was unlikely to act as a chemical transmitter in the carotid body. Lysergic acid diethylamide (LSD), methysergide and gramine, which, although not specific, are all antagonists of 5-HT, were found to block the vascular response to 5-HT but not the chemoreceptor response which suggests that the chemoreceptor response and the vascular response are mediated by different receptor types. Low doses of LSD were found to cause a marked stimulation of chemoreceptor activity. The author, however, offers no explanation for this latter phenomenon.

It is not known whether or not 5-HT is released from cells in the carotid body during chemoreceptor stimulation. The distribution of enzymes concerned in 5-HT metabolism has apparently not been studied although the carotid body is known to contain monoamineoxidase (MAO) (Lee and Mattenheimer, 1964) which is an important enzyme in the catabolism of monoamines such as 5-HT. There is, therefore, little evidence in favour of the idea that 5-HT acts as a chemical transmitter of sensory impulses in the carotid body. Nevertheless, the evidence available at the present time suggests that the carotid body contains small amounts of 5-HT and administration of 5-HT can influence chemoreceptor activity. The possibility that endogenous 5-HT has a physiological role in the initiation or modulation of afferent chemoreceptor activity cannot be excluded.

## SECTION II

### METHODS AND MATERIALS

Experiments were performed on anaesthetized cats or rabbits (details of numbers, sex, weight etc. of cats and rabbits are given in Sections III and IV respectively). The method used was essentially the same for experiments on animals of either species differing only in some details as indicated below.

### Anaesthesia

Cats were anaesthetized with sodium pentobarbitone (42 mg/kg I.P.), supplemented every 1-2 hr during the experiment by 10% of the initial dose administered I.V. Rabbits were anaesthetized with sodium pentobarbitone (30 - 50 mg/kg) or urethane (400 mg/kg) and  $\alpha$ -chloralose (6 mg/kg of a 1% solution in 0.9% saline), administered through an ear vein, with supplements as required.

### General

A cannula was inserted into the trachea low in the neck. Both femoral arteries were cannulated, one catheter being connected to a B.P. transducer (Bell and Howell, 4-442) and the other used for withdrawing blood samples for subsequent gas analysis. The signal from the transducer was displayed on a pen-recorder (Devices, M4) and recorded on one channel of an FM tape recorder (Tandberg, 100; frequency response d.c. to 1250 Hz). Arterial blood pH,  $pO_2$  and  $pCO_2$  were measured at hourly intervals using a Radiometer gas monitor (BMS 3 with PHM 71 meter). A femoral vein was cannulated and used for drug administration. Rectal temperature was monitored and maintained at  $38 \pm 0.5^\circ C$  for cats,  $39 \pm 0.5^\circ C$  for rabbits, by a heating pad.

The carotid bifurcation region was exposed and dissected free of surrounding tissue. A cannula was inserted into the lingual

artery until its tip lay in the common carotid artery 1.5 - 2.0 cm caudal to the carotid bifurcation. This cannula was used for intracarotid (I.C.) administration of drugs to the carotid body. Figures 2.1B and 2.1A show diagrams of the gross anatomy in the carotid bifurcation region of the cat and rabbit respectively.

Animals were artificially ventilated with room air by a respiratory pump (S.R.I.), operating at 38 strokes/min for rabbits or 25 strokes/min for cats, and gallamine triethiodide (3 mg/kg I.V.) administered to paralyse spontaneous respiration. This treatment also prevented muscle contraction, either spontaneous or in response to close-arterial injections of ACh, from moving the nerve on the recording electrodes.

In most experiments, end-tidal  $\text{CO}_2$  was continuously monitored by an infra-red  $\text{CO}_2$  analyser (Med 1A; Grubb Parsons) and maintained at about 5% by appropriate adjustment of the pump stroke volume.

#### Recording of sinus nerve activity

The sinus nerve, ipsilateral to the catheterized lingual artery was identified and cut centrally. Exposed tissues were covered with warm ( $37^\circ\text{C}$ ) mineral oil. The peripheral portion of the cut sinus nerve was placed on a small moveable platform immersed in the mineral oil so as to facilitate further dissection of the nerve. The outer sheath of the nerve was partially removed and small filaments were dissected from the main nerve trunk. Afferent electrical activity in filaments of the nerve was recorded using bipolar platinum-iridium electrodes, amplified by an a.c. amplifier (Neurolog, Digitimer), displayed on an oscilloscope (Tektronix, 5103N) and recorded on one channel of the tape recorder. Chemo-



receptor units were identified by their random pattern of discharge, their increase in discharge frequency following injection of NaCN (5  $\mu$ g) into the ipsilateral common carotid artery, their increase in discharge in response to hypoxia (breathing 10% or 5% O<sub>2</sub> in N<sub>2</sub> or 100% N<sub>2</sub>), and by the inhibition of discharge in response to hyperoxia (breathing 100% O<sub>2</sub>).

In all the experiments with cats, the ganglio-glomerular nerves were cut in order to eliminate reflex effects of sympathetic activity on carotid nerve discharge (Floyd and Neil, 1952; Eyzaguirre and Lewin, 1961). A similar operation in rabbits proved to be technically difficult since the ganglio-glomerular nerve in the rabbit is not discrete, as are the corresponding nerves in the cat, but joins the sinus nerve before reaching the carotid sinus (see Figure 2.1A). It is therefore difficult to cut the ganglio-glomerular nerve without damaging the sinus nerve. Elimination of sympathetic effects was however accomplished in four rabbits, without noticeable damage to the sinus nerve, by cutting the ganglio-glomerular nerve in two animals and by removing the superior cervical ganglion in another two.

#### Drug administration

Drugs used in the present study fall into two categories:

- a) test drugs which were used to evoke a chemoreceptor response, eg. ACh or DA, and
- b) modifying drugs which were used to modify responses to test drugs, eg. atropine or  $\alpha$ -flupenthixol.

Table 2.1 lists the drugs used in each category.



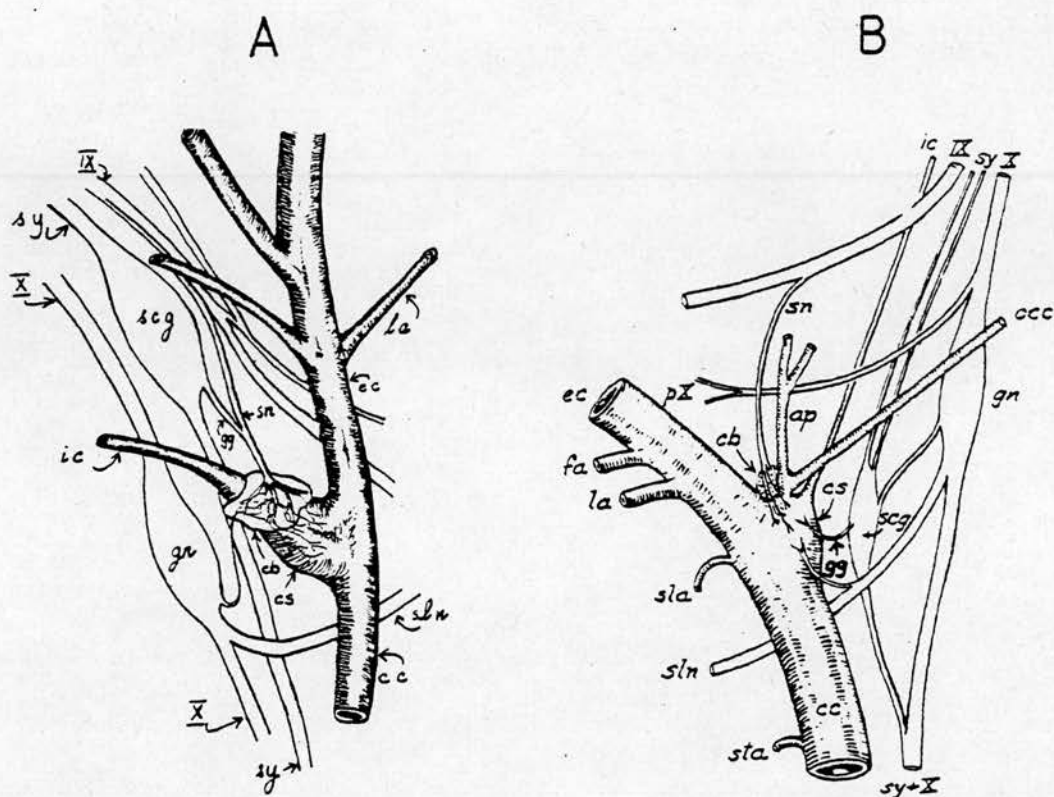


Figure 2.1

The right carotid bifurcation of the rabbit (A) and the left carotid bifurcation of the cat (B) and associated nerves. Abbreviations for both A and B are : ap, ascending pharyngeal artery; cb, carotid body; cs, carotid sinus; ec, external carotid artery; fa, facial artery; gn, ganglion nodosum; gg, ganglioglomerular nerves; ic, internal carotid artery; la, lingual artery; occ, occipital artery; pX, pharyngeal branch of X; scg, superior cervical ganglion; sla, superior laryngeal artery; sln, superior laryngeal nerve; sn, sinus nerve; sta, superior thyroid artery; sy, cervical sympathetic trunk; IX, glossopharyngeal nerve; X, vagus nerve. Part B of the figure is taken from Adams (1958).

Table 2.1:

Test drugs	Modifying drugs
acetylcholine	apomorphine
D-amphetamine	atropine
bethanechol	benztropine
dopamine	$\alpha$ -flupenthixol
5-hydroxytryptamine	haloperidol
methacholine	mecamylamine
noradrenaline	nomifensine
sodium cyanide	pargyline
suberyldicholine	phenoxybenzamine
sodium nitrite	physostigmine
sodium nitroprusside	propranolol

I.C. injections of test drugs were made in a volume of 0.1 ml and modifying drugs in a volume of 0.1 - 0.2 ml and the catheter (dead space = 0.1 ml) flushed with 0.2 ml of modified Locke solution (composition given below) which had been bubbled with a 5% CO<sub>2</sub>/95% air gas mixture. Bubbling the Locke solution in this way prevented a "flush response" which is sometimes observed following I.C. injections of Locke solution (Eyzaguirre and Nishi, 1974). Injections were made at the peak of the inspiratory phase of the respiratory cycle and completed over one respiratory cycle. A period of 3 - 5 min was allowed between injections of test drugs, unless otherwise indicated in the text. I.V. injections of drugs were made in a volume of 0.2 - 1.0 ml and the catheter flushed with 0.5 ml 0.9% (w/v) saline. In addition to test drugs, chemoreceptor responses were obtained in some experiments to CO<sub>2</sub> or hypoxia. Chemoreceptor responses to CO<sub>2</sub> were obtained by injecting 0.1 - 0.3 ml I.C. of

hypercapnic Locke solution (i.e. modified Locke solution which had been bubbled for 2 min with 100% CO<sub>2</sub> (pCO<sub>2</sub> > 160 mm Hg, arithmetic mean pH ± standard error = 5.85 ± 0.03)). In experiments performed early in the present study, responses to hypoxia were obtained by ventilating the lungs of the experimental animal with 100% N<sub>2</sub> for 2 min. In later experiments a less severe stimulus of 5 or 10% O<sub>2</sub> in N<sub>2</sub> was used.

Control injections of modified Locke, i.e. 0.1 ml Locke equilibrated with room air injected I.C. and the catheter flushed with 0.2 ml Locke bubbled with 5% CO<sub>2</sub> in air, were made in every experiment.

#### Data analysis

Data were analysed by the method of McQueen (1977) which may be summarized as follows. Recordings of nerve activity were selected for data analysis according to the following criteria:

- a) the amplitude of the action potentials to be counted (chemoreceptor units) was sufficiently different from that of other nerve potentials that they could be clearly distinguished throughout the course of an experiment, and
- b) the population of units being counted in a recording (maximum 6 but usually 1 - 3) remained constant throughout the course of an experiment.

Individual units, which had a period of at least 7 msec between successive spikes, were identified by the shape of the action potential which was usually characteristic of a given unit and remained consistent throughout the course of an experiment.

Many recordings of afferent activity were rejected because one or

other of these criteria were not satisfied. This is particularly true of recordings obtained from the rabbit sinus nerve where it was found especially difficult to separate afferent chemoreceptor activity in the nerve from other activity, e.g. baroreceptor activity. Afferent activity in the rabbit sinus nerve is discussed further in Appendix I.

The output of the tape channel on which action potentials were recorded was fed into a pulse height discriminator, the upper and lower levels at which the discriminator operated being indicated by z-axis modulation. The analogue output, which had been stored for 1 sec, was fed to a digital volt meter (Schlumberger, A210) coupled to a data transfer unit (Schlumberger, 3240) which drove an Addo 5 punch.

Average discharge ( $\bar{x}$ , counts/sec) in the 'control period' prior to administration of a test drug, generally 10 - 20 sec, was computed (PDP8 computer, Digital Equipment Corporation) from the punched tape. The average ( $\bar{x}$ ) and total counts ( $\Sigma x$ ) were calculated for each response after its duration ( $t$  sec) had been determined from a histogram of the response, displayed by the computer on an x-y plotter (Complot, Houston Instruments). Responses to test drugs were expressed as the absolute difference in discharge ( $\Delta \Sigma x$ ) following drug administration where:

$$\Delta \Sigma x = \Sigma x (\text{response}) - \Sigma x (\text{control})$$

and

$$\Sigma x (\text{control}) = \bar{x} (\text{control}) \times t$$

Data were expressed in this way since measurement of  $\Delta \Sigma x$  takes into account any variation in  $t$  as well as any change in  $\bar{x}$  (see McQueen, 1977).





The value of  $\bar{x}$  (control) for the 10 - 20 sec period preceding administration of a test drug was also used to calculate the overall spontaneous discharge frequency in animals breathing room air before and after administration of a modifying drug. This value is given by,

$$\text{overall } \bar{x} = \left( \sum_{i=1}^n \bar{x}(\text{control})_i \right) \left( \frac{1}{n} \right)$$

where  $n$  was the number of injections of test drugs given before or after administration of a modifying drug. In other words, overall  $\bar{x}$  - the average spontaneous chemoreceptor discharge frequency in animals breathing room air - was the arithmetic mean of the values of  $\bar{x}$  (control) for all the test drugs administered under a particular set of experimental conditions, i.e. before or after administration of a modifying drug. Since 8 - 12 injections of test drugs were normally given before and after administration of a modifying drug, the values for spontaneous chemoreceptor discharge frequency in animals breathing room air, quoted in Sections III and IV, refer to the average discharge frequency in a period of 30 - 45 min preceding or following administration of a modifying drug. Where data were available from more than one experiment values of overall  $\bar{x}$ , calculated as above, were pooled and expressed as the arithmetic mean  $\pm$  standard error.

Whenever possible, responses were obtained to a range of doses of a test drug and  $\Delta \Sigma x$  was plotted against  $\log_{10}$  dose. The range of doses over which the relationship was approximately linear was determined and a straight line was fitted to the points in the linear portion of the dose-response curve using the method of least squares. An estimate of the effect of a modifying drug on responses

to test drugs was obtained by comparing the dose-response line obtained before administration of the modifying drug to that obtained after. A response in the central region of the control dose-response line, of magnitude R, was selected arbitrarily and the dose of test drug,  $D_1$ , required to produce this response was calculated using the equation for a straight line,  $y = mx + c$  where m is the gradient and c is the intercept with the y-axis. The dose of a test drug,  $D_2$ , required to produce a response of magnitude R after administration of a modifying drug was then calculated. The ratio,  $D_1/D_2$  was called the dose-ratio and provided a quantitative measure of the effect of a modifying drug on responses to test drugs. It should be stressed that dose-ratios, calculated in this way, are not equivalent to pharmacological dose-ratios obtained using isolated tissues *in vitro* from which affinity constants may be calculated since the concentration of a given drug in the carotid body is unknown but are employed here only as a method of quantifying the effects of modifying drugs on chemoreceptor responses to test drugs. Dose-ratios were calculated only if the dose-response lines obtained before and after administration of a modifying drug were approximately parallel.

When the same dose of a modifying drug was used in three or more experiments, the dose-ratios from the different experiments were pooled and data are presented as the geometric mean of the dose ratios given by:

$$\sqrt[n]{P_1 \times P_2 \times \dots \times P_n}$$

where  $P_1 \dots P_n$  are the dose-ratios calculated in n experiments.

The median and the range (highest value minus lowest value) are also



quoted to indicate the scatter of individual ratios about the mean value. When the same dose of a modifying drug was used in only one or two experiments, typical dose-response data from one or other experiment is presented to illustrate the effect of the drug. This is especially true in Section IV in which the effects of modifying drugs were studied over a wide range of doses and consequently less data are available for individual doses.

Linearly related dose-response data for test drugs was obtained whenever possible. However, owing to the limited life-span of a given recording it was considered expedient, in some cases, to administer test drugs in single doses before and after administration of a modifying drug. This situation arose in experiments in which it was desirable to study the effect of several different test drugs before and after administration of a modifying drug and insufficient time was available to construct dose-response lines for all the test drugs. The dose of test drug selected for such single dose studies was one which, from experience, would be expected to produce a response which was sub-maximal but well above the threshold dose required to produce an effect. The usefulness of data derived from single dose studies of this type was limited inasmuch as subtle changes in responsiveness were difficult to detect and dose-ratios could not be calculated. Nevertheless data of this type was considered a useful supplement to linearly-related dose-response data obtained for other test drugs in the same experiment.

### Drugs

Drugs were prepared in modified Locke solution (NaCl, 6.0 g; KCl, 0.42 g; CaCl<sub>2</sub>, 0.24 g; Tris base, 6.0 g; N-HCl, 39 ml;

distilled water to 1.0 l) excepting  $\alpha$ -flupenthixol, which was dissolved in 0.9% aqueous sodium chloride, and haloperidol, which was dissolved in 1% aqueous tartaric acid.

The drugs used were: sodium pentobarbitone (Abbott Laboratories); gallamine triethiodide (May & Baker); acetylcholine iodide, sodium cyanide, atropine sulphate, urethane (ethyl carbamate),  $\alpha$ -chloralose, sodium nitrite and physostigmine salicylate (all B.D.H.); 5-hydroxytryptamine creatinine sulphate, D-amphetamine sulphate, pargyline hydrochloride and propranolol hydrochloride (all Sigma); dopamine hydrochloride, bethanechol chloride, noradrenaline bitartrate and methacholine bromide (all Koch-Light); benztropine mesylate and mecamlamine hydrochloride (both M.S.D.);  $\alpha$ (cis)-flupenthixol dihydrochloride (Lundbeck & Co.); apomorphine hydrochloride (Macfarlan Smith); haloperidol (Janssen); sodium nitroprusside (Griffin & Tatlock); nomifensine hydrogen maleate (Hoechst); phenoxybenzamine hydrochloride (S.K.F.); suberyldicholine di-iodide (kindly supplied by Dr. A. Ungar, Department of Pharmacology, University of Edinburgh).

### SECTION III

#### THE EFFECTS OF DOPAMINE, 5-HYDROXYTRYPTAMINE AND NORADRENALINE ON CHEMORECEPTOR ACTIVITY IN THE CAT CAROTID BODY

## INTRODUCTION

DA is present in the mammalian carotid body and a number of hypotheses have been proposed which suggest that DA acts as an endogenous inhibitor of carotid chemoreceptor activity (see Section I). The present experiments were performed in order to study the inhibitory effects of DA on chemoreceptor activity in the cat carotid body *in situ*, and also to examine the effects of drugs which influence dopaminergic systems on chemoreceptor responses to stimulants such as NaCN, ACh and hypoxia.

The carotid body also contains NA and 5-HT (see Section I). Since drugs which modify dopaminergic systems may have non-specific effects on noradrenergic and serotonergic systems, the opportunity was taken to investigate the effects of NA and 5-HT on carotid chemoreceptor activity in this species and to determine what effect drugs which modify dopaminergic systems have on chemoreceptor responses to NA and 5-HT.

## RESULTS

Experiments were performed on 37 cats (22 male and 15 female) weighing between 2.0 and 4.0 kg (mean weight  $\pm$  standard error =  $3.0 \pm 0.7$  kg). Forty-one recordings of afferent chemoreceptor activity were obtained from 34 of these animals. Animals were anaesthetized with sodium pentobarbitone as described in Section II.

### Responses to dopamine

Administration of DA (0.5 - 50  $\mu$ g I.C.) caused an immediate, short-lasting (5 - 45 sec) inhibition of chemoreceptor activity in all

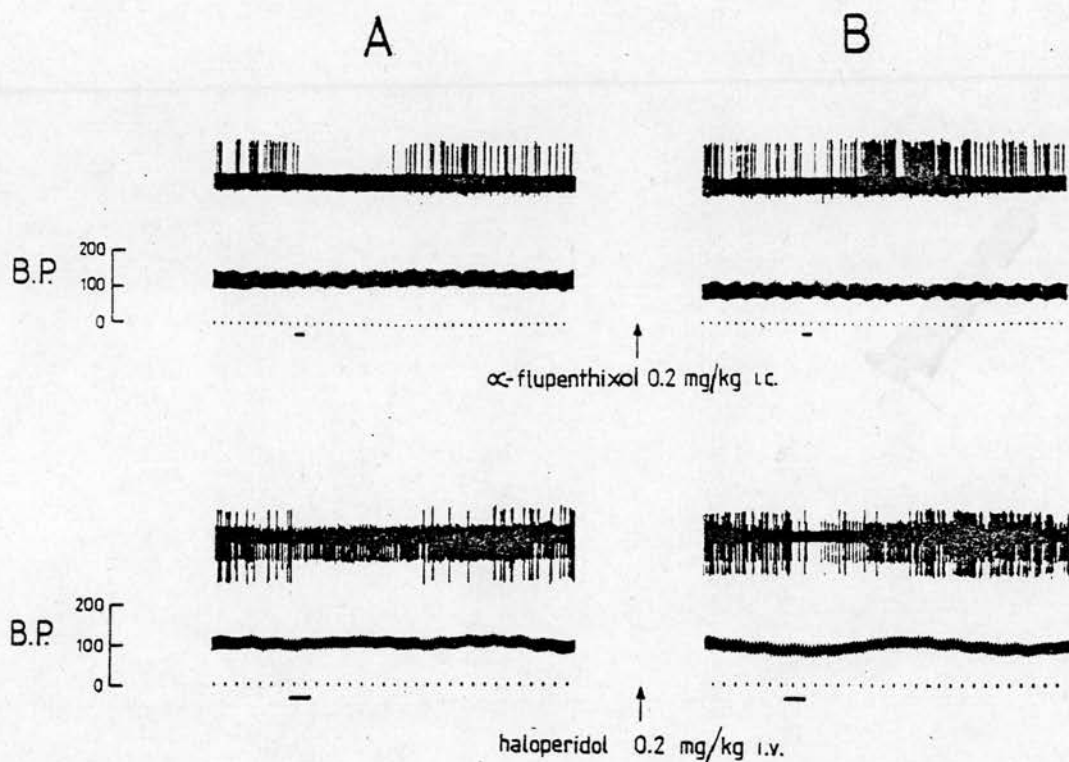


Figure 3.1

Chemoreceptor units from two experiments showing responses to DA (2.5 µg I.C.) before (A) and after (B) administration of  $\alpha$ -flupenthixol (0.2 mg /kg I.C.) (upper panels) and haloperidol (0.2 mg /kg I.V.) (lower panels). Each panel shows, from above downwards : B.P. (mm Hg), one second time marks and injection marker. The smaller unit in the lower panels is a baroreceptor unit.



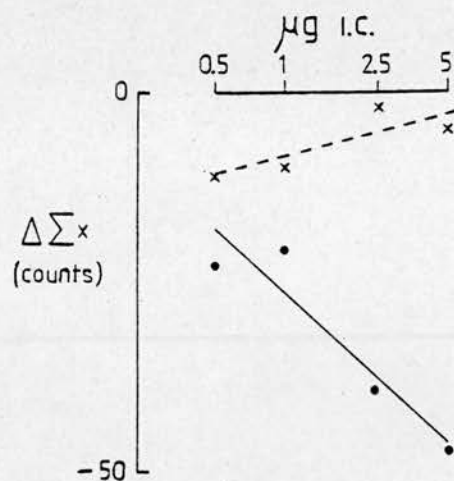


Figure 3.2

Dose-response data for DA, from a recording of one chemoreceptor unit, before (●—●) and after (x--x) administration of  $\alpha$ -flupenthixol (0.2 mg /kg I.C.). Doses are plotted on a  $\log_{10}$  scale and lines are fitted by the method of least squares.

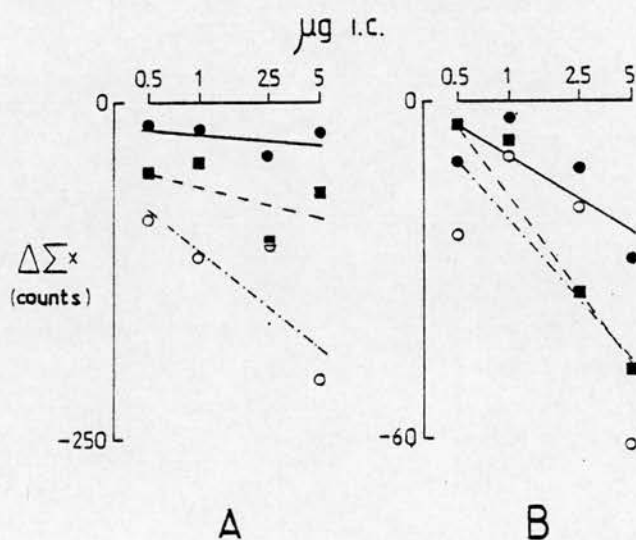


Figure 3.3

Dose-response data for DA (A) and 5-HT (B), from a recording of two chemoreceptor units, obtained during artificial ventilation of the experimental animal with air (●—●), 17.5 %  $O_2$  in  $N_2$  (■--■) and 15 %  $O_2$  in  $N_2$  (○---○). Arterial  $pO_2$  was 92 mm Hg, 61 mm Hg and 45 mm Hg and pH was 7.33, 7.36 and 7.34 respectively. Doses are plotted on a  $\log_{10}$  scale and lines fitted to the data by the method of least squares.

the experiments (eg. see Figure 3.1A), the magnitude of the inhibition being dose-dependent over the range 0.5 - 5  $\mu\text{g}$  I.C. (see Figure 3.2). There was a tendency for DA to cause a delayed or secondary increase in discharge, especially at higher doses (10 - 50  $\mu\text{g}$  I.C.) which curtailed the primary inhibition and gave inconsistent responses. In view of this observation, the present study was confined to low doses of DA ( $\leq 10$   $\mu\text{g}$  I.C.) with which secondary excitatory effects were obtained only infrequently and which gave more consistent inhibitory responses.

In three experiments the effect of hypoxia on the response to DA was studied. It was found that the magnitude of the inhibitory response to DA was inversely proportional to arterial  $pO_2$ , i.e. the magnitude of the response was proportional to the background frequency of spontaneous chemoreceptor activity against which the inhibition was measured (see Figure 3.3A).

There was a slight increase in mean arterial B.P. which commenced about 10 sec after an I.C. injection of high doses of DA ( $\geq 10$   $\mu\text{g}$  I.C.). With lower doses there was no obvious change in B.P.

### Effects of dopamine antagonists

#### 1. $\alpha$ -Flupenthixol

$\alpha$ -Flupenthixol is a potent DA antagonist (Moller Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland and Christensen, 1973; Miller, Horn and Iversen, 1974) which has very little anticholinergic activity (Iversen, 1975). The effect of  $\alpha$ -flupenthixol on chemoreceptor activity was studied in nine experiments.

Low doses of  $\alpha$ -flupenthixol (0.05 mg/kg I.C.) reduced, and higher doses (0.2 - 1.0 mg/kg I.C.) abolished DA-induced inhibition

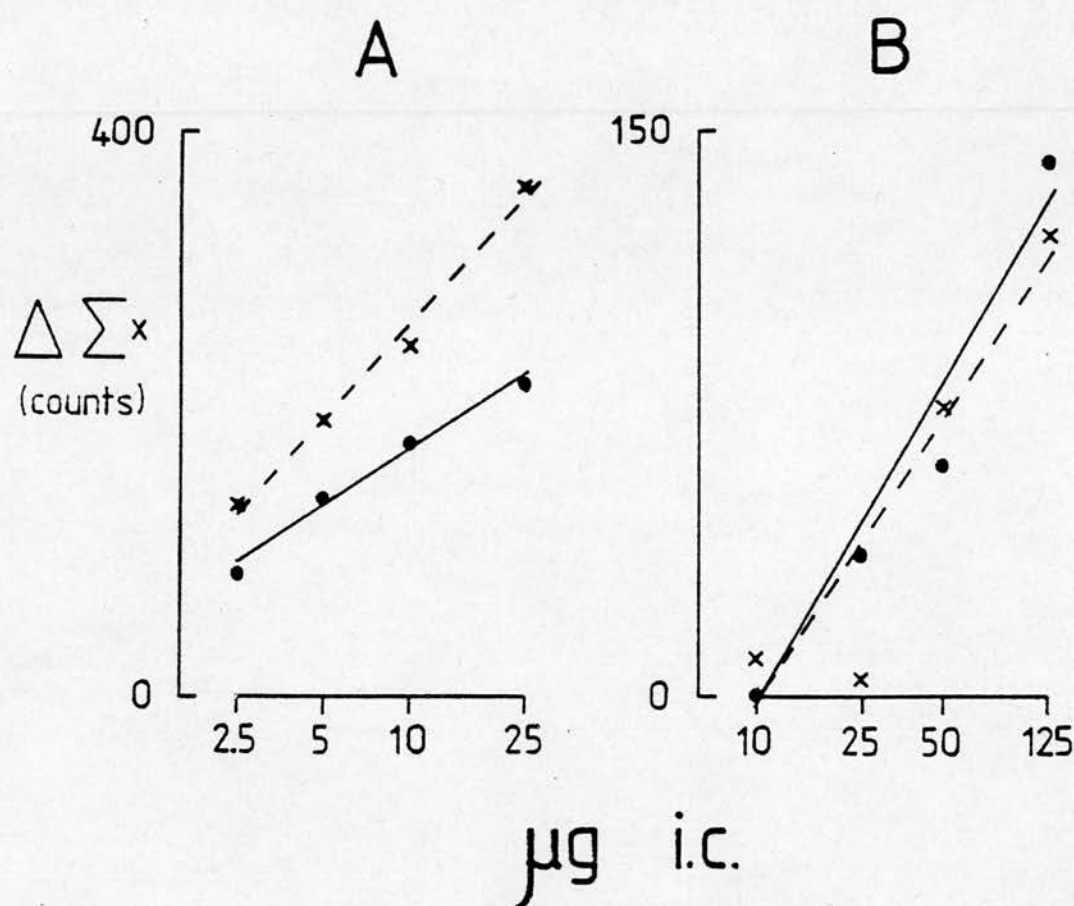
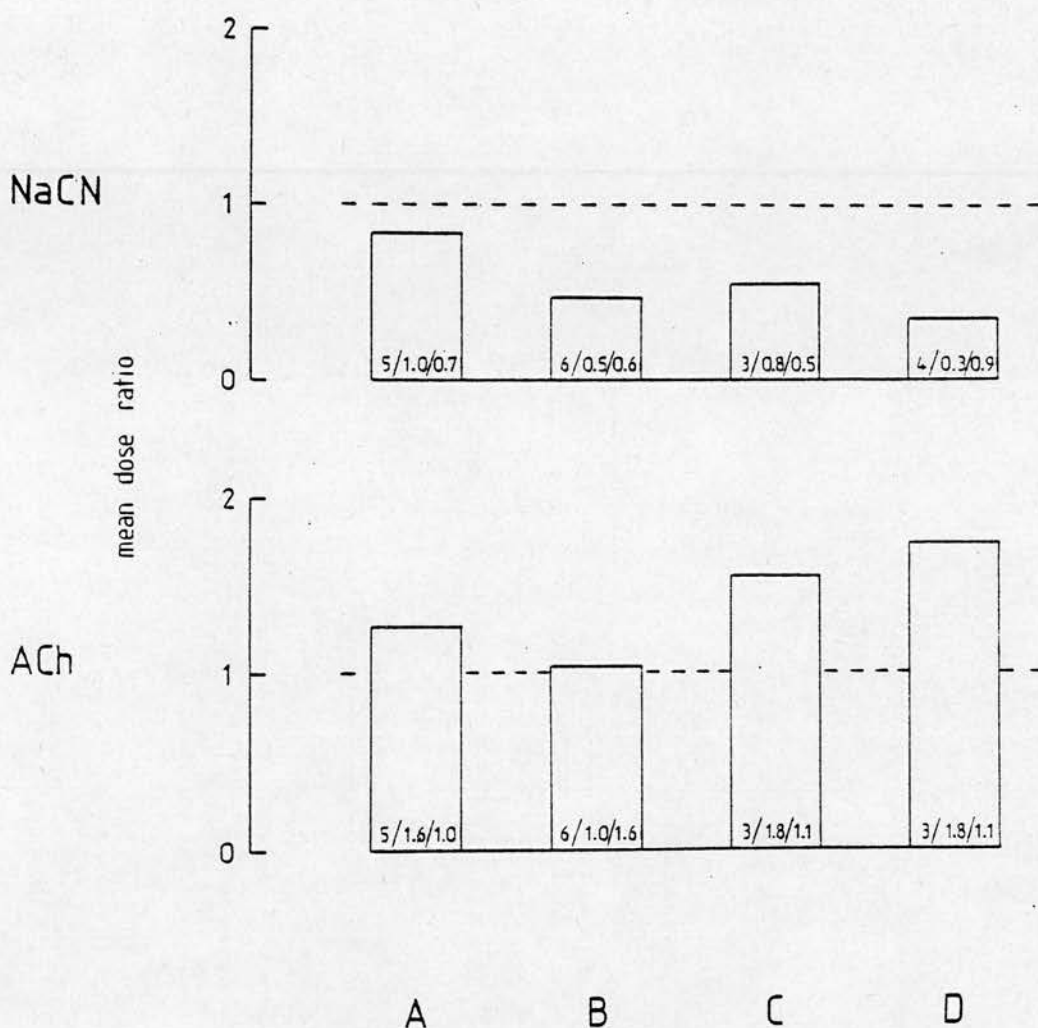


Figure 3.4

Dose-response data for NaCN (A) and ACh (B), from a recording of two chemoreceptor units, before ( $\bullet$ — $\bullet$ ) and after ( $\times$ — $\times$ ) administration of  $\alpha$ -flupenthixol (0.2 mg /kg I.C.). Doses are plotted on a  $\log_{10}$  scale and lines fitted by the method of least squares.



**Figure 3.5**

Pooled dose-ratio data showing the effects of  $\alpha$ -flupenthixol, in doses of 0.05 mg /kg I.C. (A), 0.2 mg /kg I.C. (B), 0.5 mg /kg I.C. (C) and 1.0 mg /kg I.C. (D), on chemoreceptor responses to NaCN and ACh. Data are presented as the geometric mean dose-ratio. The dashed line represents a dose-ratio of one. The number of experiments (n), the median value (n) and the range of values (r) are inset in each column as n/m/r.

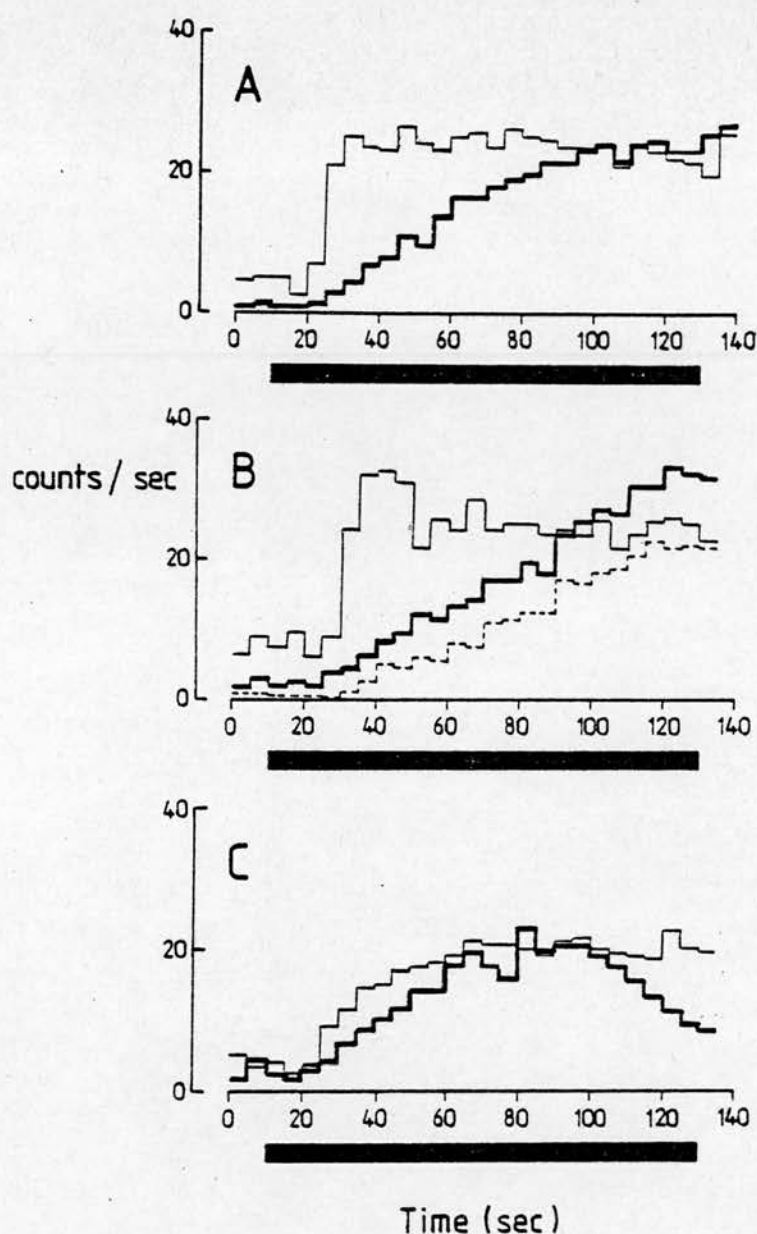


Figure 3.6

Response of carotid chemoreceptors to 120 sec of hypoxia (breathing 100%  $N_2$ ). The top panel (A) shows the response before (—) and after (---) administration of  $\alpha$ -flupenthixol (0.2 mg /kg I.C.). The centre panel (B) shows the response before (—) and after (---) administration of apomorphine (0.2 mg /kg I.C.) and after (----) subsequent administration of  $\alpha$ -flupenthixol (0.2 mg /kg I.C.). The bottom panel (C) shows the response before (—) and after (---) administration of haloperidol (0.2 mg /kg I.V.). Recordings were of two, two and one chemoreceptor units in A, B and C respectively. Each panel shows from above downwards : discharge frequency (averaged over 5 sec intervals), time and period of hypoxia (horizontal black bar).



of chemoreceptor activity (see Figures 3.1, 3.2). There was an increased tendency for DA to produce excitatory effects following administration of  $\alpha$ -flupenthixol, especially when high doses (0.5 or 1.0 mg/kg I.C. additional to 0.05 and 0.2 mg/kg I.C. respectively) of this blocking drug were used (see Figure 3.1).

In order to make a quantitative estimate of the effects of  $\alpha$ -flupenthixol on chemoreceptor responses to NaCN and ACh, dose-response data were obtained before and after administration of  $\alpha$ -flupenthixol (see Figure 3.4) and dose-ratios (see Section II) calculated from the dose-response lines (see Figure 3.5). Low doses of  $\alpha$ -flupenthixol (0.05 mg/kg I.C.) had no effect on responses to NaCN while higher doses (0.2 - 1.0 mg/kg I.C.) caused potentiation (see Figures 3.4, 3.5). Chemoreceptor responses to ACh were not appreciably affected by low doses of  $\alpha$ -flupenthixol (0.05 or 0.2 mg/kg I.C.) but were reduced by higher doses (0.5 or 1.0 mg/kg I.C.) (see Figures 3.4, 3.5). Unfortunately it was not possible to calculate dose-ratios for DA since, after administration of  $\alpha$ -flupenthixol, the slope of the dose-response line became positive, reflecting the tendency for DA to evoke excitatory responses under these conditions (see Figure 3.2).

The rate of increase of spontaneous chemoreceptor discharge during hypoxia (breathing 100% N<sub>2</sub>) was greatly increased following administration of  $\alpha$ -flupenthixol although the maximum discharge frequency reached was not appreciably different from control values (see Figure 3.6A). The frequency of spontaneous discharge in animals breathing room air was little affected by  $\alpha$ -flupenthixol except after high doses (1.0 mg/kg I.C.) when there was a slight increase in frequency (see Table 3.1)

TABLE 3.1: Spontaneous chemoreceptor discharge frequency (arithmetic mean ( $\bar{x}$ )  $\pm$  standard error; counts/sec) in animals breathing room air, before and after administration of  $\alpha$ -flupenthixol

No. of experiments	Dose of $\alpha$ -flupenthixol (mg/kg I.C.)	$\bar{x}$ before	$\bar{x}$ after
5	0.05	$3.7 \pm 1.3$	$4.3 \pm 1.0$
6	0.20	$4.7 \pm 0.8$	$5.9 \pm 1.1$
3	0.50	$3.0 \pm 0.6$	$3.4 \pm 0.6$
4	1.00	$6.8 \pm 1.5$	$10.3 \pm 1.3$

## 2. Haloperidol

Haloperidol blocks the action of DA at dopaminergic synapses in the C.N.S. (Bunney, Walters, Roth and Aghajanian, 1973). The effects of haloperidol, in cumulative doses of 0.2, 0.5 and 1.0 mg/kg I.V., on chemoreceptor activity were studied in one experiment and the results obtained were qualitatively similar to those obtained with  $\alpha$ -flupenthixol. The lowest dose (0.2 mg/kg I.V.) had little effect, while higher doses (0.5 and 1.0 mg/kg I.V.) reduced, but did not abolish, the inhibitory action of DA (see Figure 3.1), enhanced the response to NaCN (see Figure 3.7A) and slightly reduced that to ACh (see Figure 3.7B). The rate of increase of spontaneous chemoreceptor discharge frequency during hypoxia (breathing 100% N<sub>2</sub>) was slightly increased following administration of haloperidol although the maximum discharge frequency reached was similar to control values (see Figure 3.6C). Spontaneous discharge frequency in animals breathing room air was 2.1 counts/sec before and 4.4 counts/sec after 0.2 mg/kg I.V., 3.1 counts/sec after 0.5 mg/kg I.V. and 2.1 counts/sec after the final dose of 1.0 mg/kg I.V. of haloperidol.

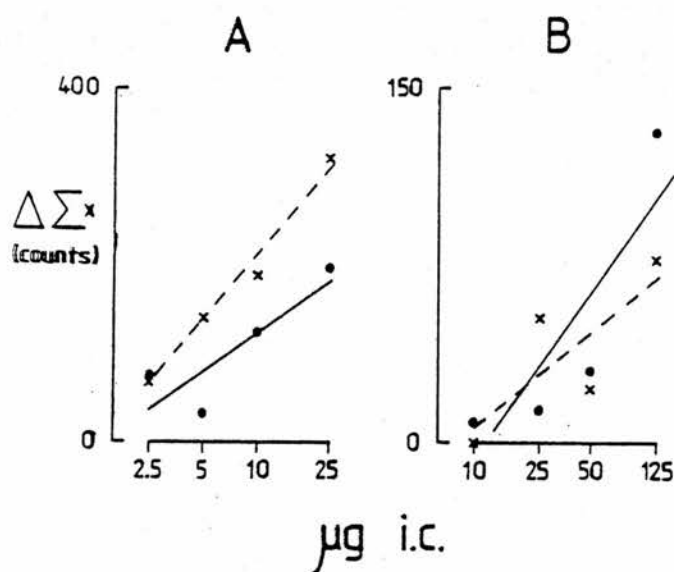


Figure 3.7

Dose-response data for NaCN (A) and ACh (B), from a recording of one chemoreceptor unit, before (●—●) and after (x--x) administration of haloperidol (1 mg /kg I.V.). Doses are plotted on a log<sub>10</sub> scale and lines fitted by the method of least squares.

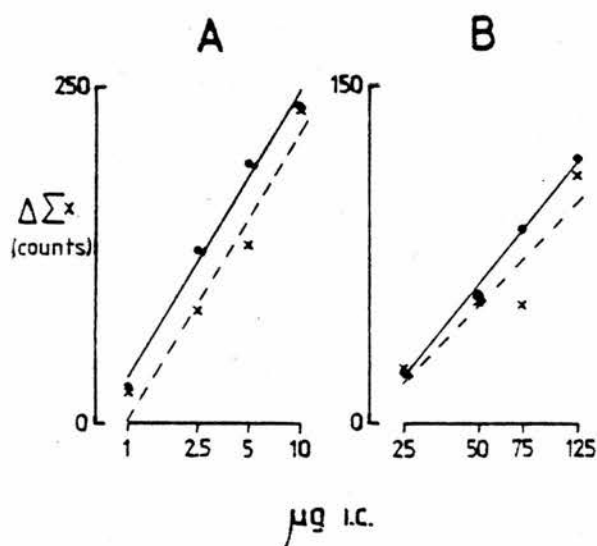


Figure 3.8

Dose-response data for NaCN (A) and ACh (B), from a recording of three chemoreceptor units, before (●—●) and after (x--x) administration of apomorphine (0.2 mg /kg I.C.). Doses are plotted on a log<sub>10</sub> scale and lines fitted by the method of least squares.

## Substances which mimic dopamine

### 1. Apomorphine

Apomorphine is a DA receptor agonist (Anden, Rubenson, Fuxe and Hokfelt, 1967) which has been shown to mimic the effects of DA at several sites (Ernst, 1969).

The effects of apomorphine, in cumulative doses of 0.05 and 0.2 mg/kg I.C., on chemoreceptor activity were studied in two experiments. Apomorphine caused a depression of spontaneous chemoreceptor activity lasting up to 45 min, spontaneous discharge frequency being  $3.0 \pm 0.4$  counts/sec before and  $1.0 \pm 0.1$  counts/sec after 0.05 mg/kg I.C. and  $1.4 \pm 0.5$  counts/sec after the additional dose of 0.2 mg/kg I.C. Dose-response data to NaCN and ACh were obtained while spontaneous discharge frequency was still depressed. Responses to both stimulants were slightly reduced (see Figure 3.8). Although background discharge frequency was reduced following administration of apomorphine, DA could still evoke an inhibitory response. The rate of increase of spontaneous discharge frequency during hypoxia was depressed following administration of apomorphine, an effect which was reversed by subsequent administration of  $\alpha$ -flupenthixol (see Figure 3.6B).

### 2. D-Amphetamine

D-Amphetamine causes release of DA from dopaminergic neurons in the C.N.S. (Bunney, Aghajanian and Roth, 1973). The effects of low doses (5 - 50  $\mu$ g I.C.) of amphetamine on chemoreceptor activity were studied in five experiments and the effects of a high dose (0.25 mg/kg I.C.) in one experiment.

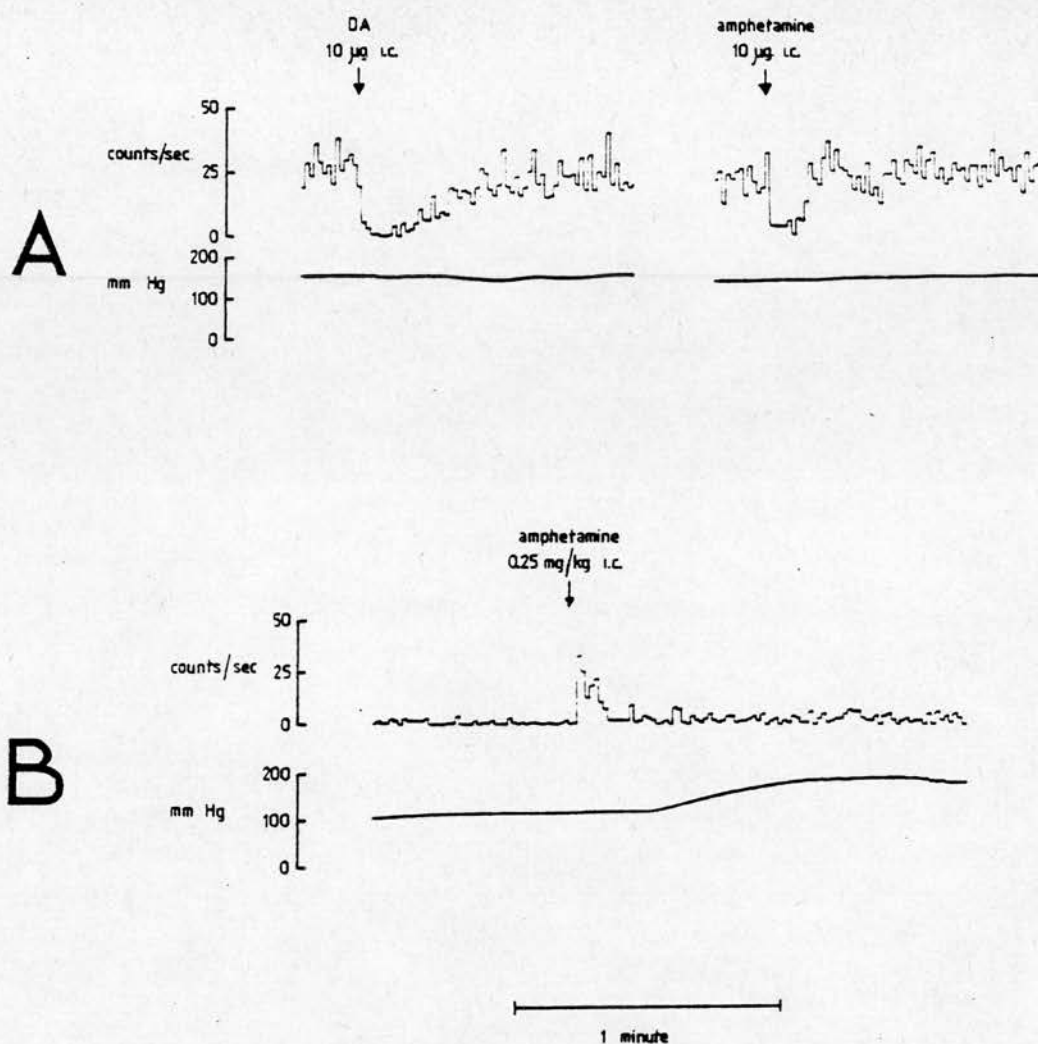


Figure 3.9

The upper two panels (A) show chemoreceptor responses from one experiment (four units) to low doses of DA and amphetamine. The lower panel (B) shows the chemoreceptor response, obtained in a second experiment (two units) to a high dose of amphetamine (0.25 mg /kg I.C.). Each panel shows, from above downwards : discharge frequency and mean arterial B.P. The time bar refers to all three panels. Injections of DA or amphetamine were made at the arrows.



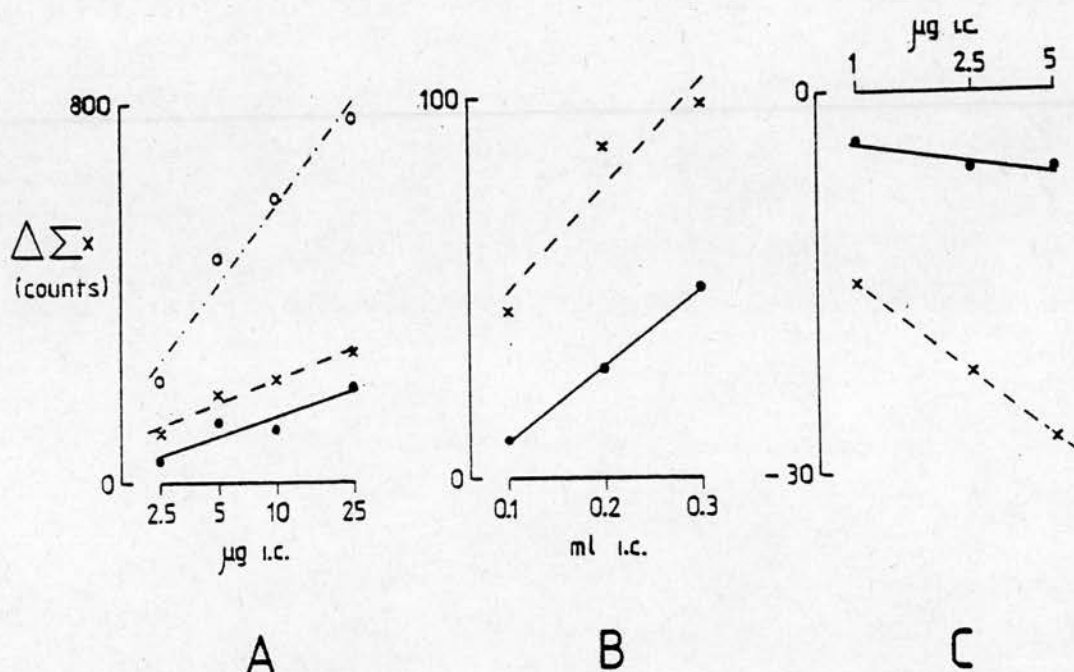
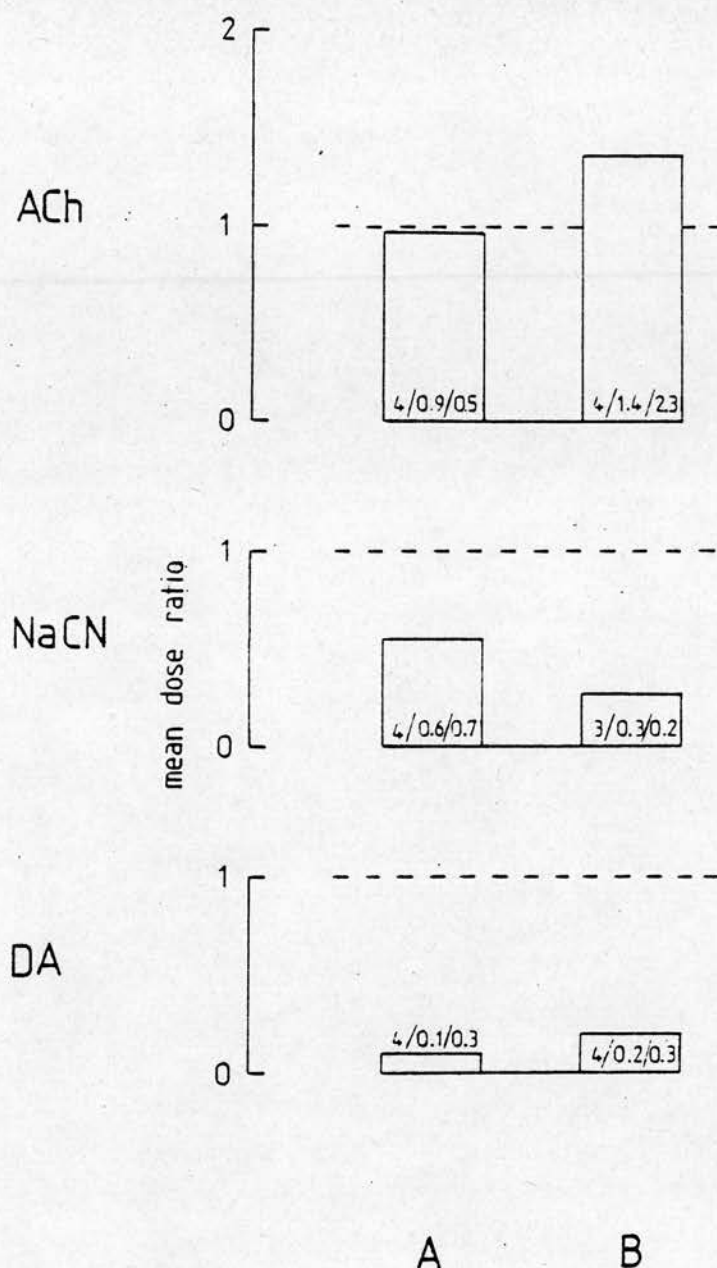


Figure 3.10

Panel A shows dose-response data for NaCN, from a recording of one chemoreceptor unit, before (●—●) and after (x---x) administration of benztropine (0.25 mg /kg I.C.) and after (o---o) subsequent administration of  $\alpha$ -flupenthixol (0.5 mg /kg I.C.). Panel B shows dose-response data for hypercapnic Locke solution, from a recording of one chemoreceptor unit, before (●—●) and after (x---x) administration of benztropine (0.5 mg /kg I.C.). Panel C shows dose-response data for DA, from a recording of one chemoreceptor unit, before (●—●) and after (x---x) administration of benztropine (0.25 mg /kg I.C.). Doses in A and C are plotted on a  $\log_{10}$  scale. Lines were fitted to the data by the method of least squares.



**Figure 3.11**

Pooled dose-ratio data showing the effect of benztropine in doses of 0.25 mg /kg I.C. (A) and 0.5 mg /kg I.C. (B) on chemoreceptor responses to ACh, NaCN and DA. Data are presented as for Fig. 3.5.

The chemoreceptor response to low doses of amphetamine was qualitatively similar to that produced by low doses of DA, i.e. inhibition (see Figure 3.9A). The inhibitory response to amphetamine, however, was not as marked as that observed following low doses of DA, did not appear to be dose-related, and was less consistent. The higher dose of amphetamine (0.25 mg/kg I.C.) evoked a transient stimulation of chemoreceptor activity and an increase in arterial B.P. (see Figure 3.9B).

### Effects of dopamine uptake blockers

#### 1. Benztropine

Benztropine is an anticholinergic agent which is, in addition, a potent blocker of DA uptake (Coyle and Snyder, 1969; Horn, Coyle and Snyder, 1971). The effect of cumulative doses of 0.25 and 0.5 mg/kg I.C. of benztropine on chemoreceptor activity was studied in four experiments.

Benztropine enhanced DA-induced inhibition of chemoreceptor activity (see Figure 3.10C) and augmented responses to NaCN (see Figure 3.10A). The chemoreceptor response to ACh was unaffected by a dose of 0.25 mg/kg I.C. but was reduced following administration of the additional dose of 0.5 mg/kg I.C. Dose ratios summarising the effects of benztropine on chemoreceptor responses to DA, NaCN and ACh are shown in Figure 3.11.

Mean arterial B.P. was reduced by approximately 15 - 20 per cent following administration of an initial dose of 0.25 mg/kg I.C. of benztropine but was not further modified by an additional dose of 0.5 mg/kg I.C. The depressor response to ACh was also reduced by benztropine.

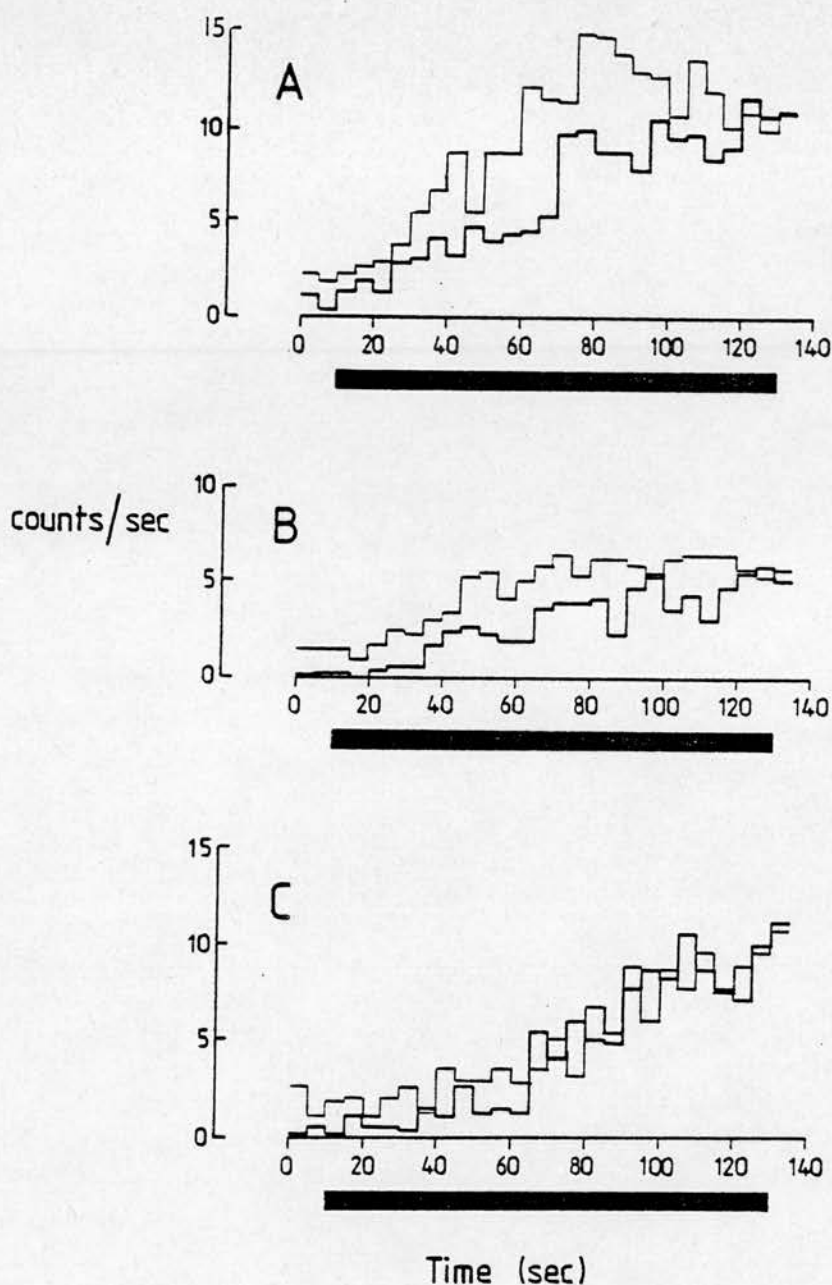


Figure 3.12

Response of carotid chemoreceptors to 120 sec of hypoxia (breathing 5% O<sub>2</sub> in N<sub>2</sub> in A and C and 10% O<sub>2</sub> in N<sub>2</sub> in B). The top panel (A) shows the response before (—) and after (---) administration of benztropine (0.25 mg /kg I.C.). The centre panel (B) shows the response before (—) and after (---) administration of nomifensine (0.2 mg /kg I.C.). The bottom panel (C) shows the response before (—) and after (---) administration of pargyline (2.5 mg /kg I.C.). Recordings were of two, one and three chemoreceptor units in A, B and C respectively. Each panel shows, from above downwards : discharge frequency (averaged over 5 sec intervals), time and period of hypoxia (horizontal black bar).

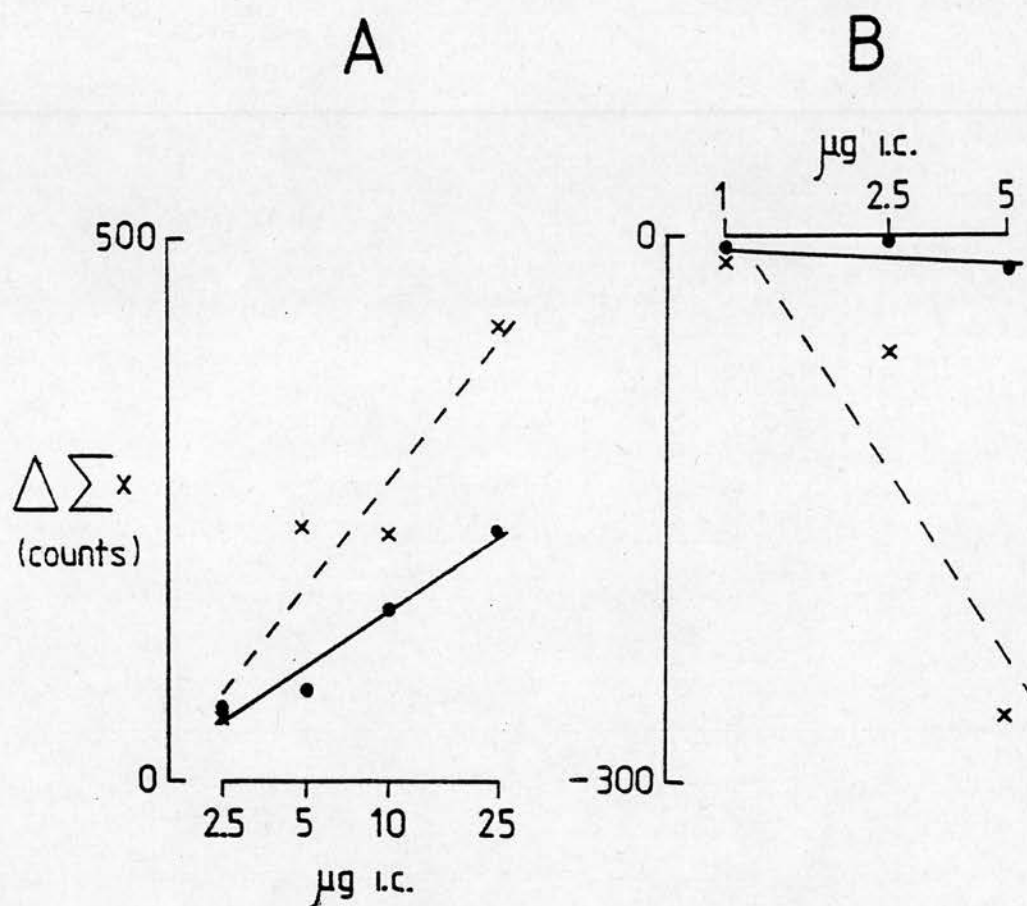
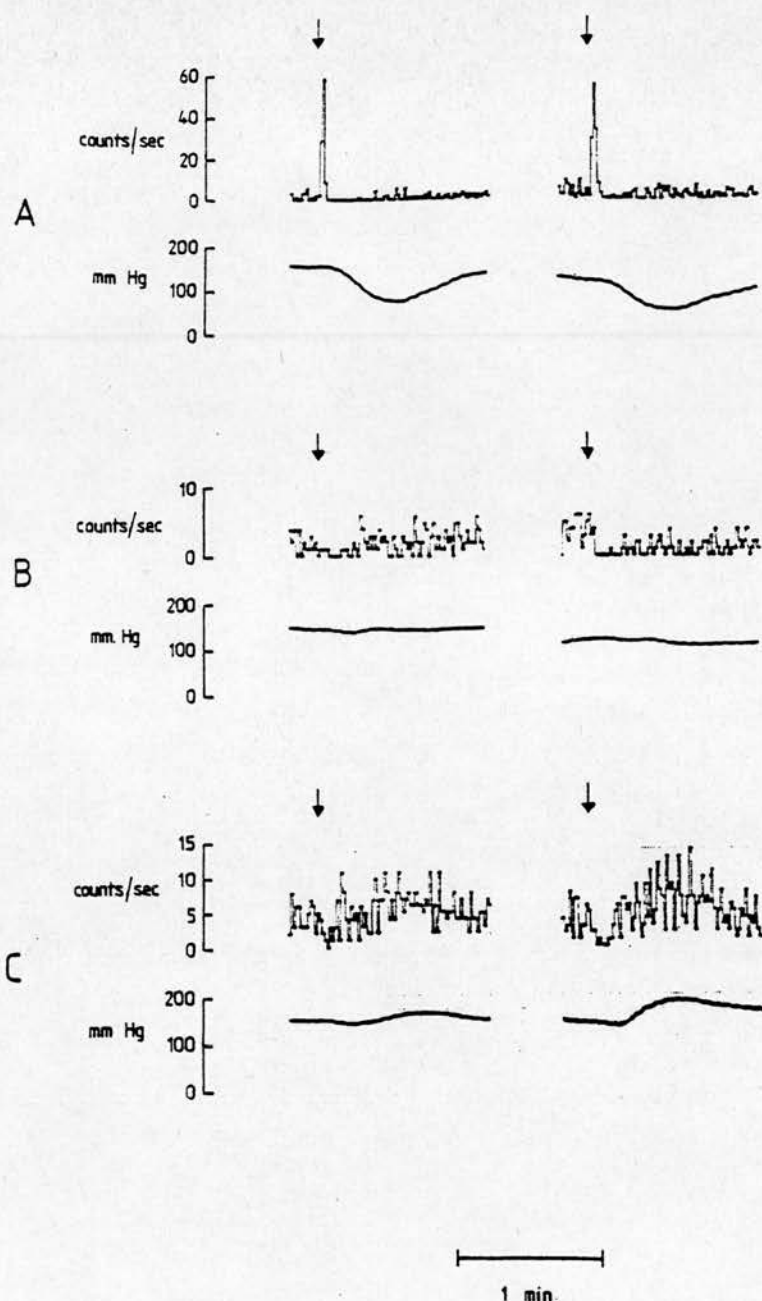


Figure 3.13

Dose-response data for NaCN (A) and DA (B) before (●—●) and after (x—x) administration of nomifensine (0.4 mg /kg I.C. in A and 0.1 mg /kg I.C. in B). Recordings were of three and four chemoreceptor units in A and B respectively. Doses are plotted on a  $\log_{10}$  scale and lines fitted by the method of least squares.





**Figure 3.14**

Responses of carotid chemoreceptors (three units) to ACh (50  $\mu$ g I.C.) (A), DA (5  $\mu$ g I.C.) (B) and NA (10  $\mu$ g I.C.) (C) before (left-hand panels) and after (right-hand panels) administration of nomifensine (0.4 mg/kg I.C.). Each panel shows, from above downwards: discharge frequency and mean arterial B.P. Injections of ACh, DA or NA were made at the arrows. The time bar refers to all six panels.

The chemoreceptor response to hypoxia (breathing 5% O<sub>2</sub> in N<sub>2</sub>) was increased following administration of benztropine (see Figure 3.12A). The response to I.C. injection of hypercapnic Locke solution (0.1 - 0.3 ml) was also increased (see Figure 3.10B). Spontaneous discharge frequency in animals breathing room air was  $1.3 \pm 0.8$  counts/sec before and  $2.6 \pm 1.1$  counts/sec after I.C. injection of 0.25 mg/kg of benztropine and  $2.4 \pm 0.3$  counts/sec after an additional dose of 0.5 mg/kg. Administration of  $\alpha$ -flupenthixol (0.5 mg/kg I.C.) subsequent to benztropine increased spontaneous chemoreceptor discharge to  $6.8 \pm 1.8$  counts/sec and further augmented responses to NaCN (see Figure 3.10A) but did not further modify responses to ACh.

## 2. Nomifensine

Nomifensine is a non-tricyclic antidepressant drug (Hoffmann, 1973) which, unlike benztropine, has little anticholinergic activity (Hoffmann, 1977) and which is a potent blocker of DA uptake (Hunt, Kannengeisser and Raynaud, 1974). Three experiments were performed in which the effects of nomifensine in doses of 0.1 mg/kg I.C., cumulative doses of 0.1 and 0.2 mg/kg I.C. and cumulative doses of 0.1, 0.2 and 0.4 mg/kg I.C. on chemoreceptor activity were studied.

Nomifensine (0.1 - 0.4 mg/kg I.C.) enhanced the chemoreceptor response to DA (see Figure 3.13B). The lowest dose of nomifensine (0.1 mg/kg I.C.) had no effect or slightly enhanced responses to NaCN while higher doses (0.2 or 0.4 mg/kg I.C.) clearly augmented responses (see Figure 3.13A). Neither the chemoreceptor response nor the depressor response to ACh (single doses of 50  $\mu$ g I.C.) were appreciably affected by nomifensine (see Figure 3.14A). There was

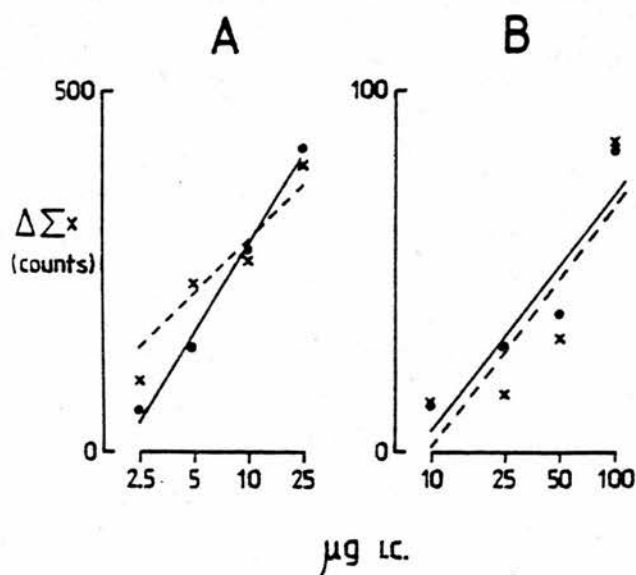


Figure 3.15

Dose-response data for NaCN (A) and ACh (B), from a recording of three chemoreceptor units, before (●—●) and after (x—x) administration of pargyline (5 mg /kg I.C.). Doses are plotted on a  $\log_{10}$  scale and lines are fitted by the method of least squares.

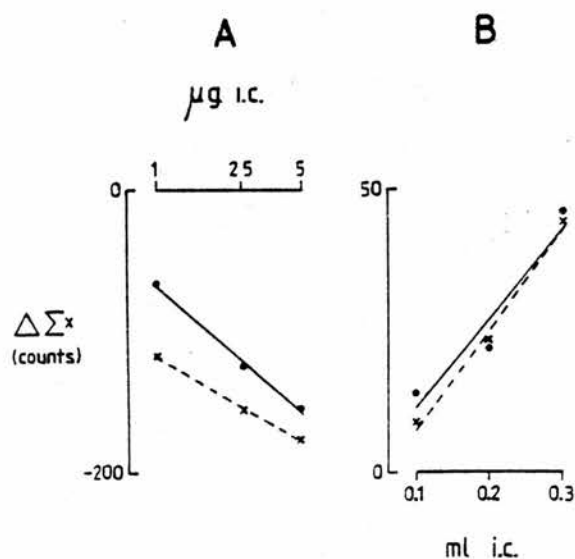


Figure 3.16

Dose-response data for DA (A) and hypercapnic Locke solution (B), before (●—●) and after (x—x) administration of pargyline (2.5 mg /kg I.C. in A and 5 mg /kg I.C. in B). Data in A and B are from recordings of two and one chemoreceptor units respectively. Doses in A are plotted on a  $\log_{10}$  scale. Lines were fitted to the data by the method of least squares.

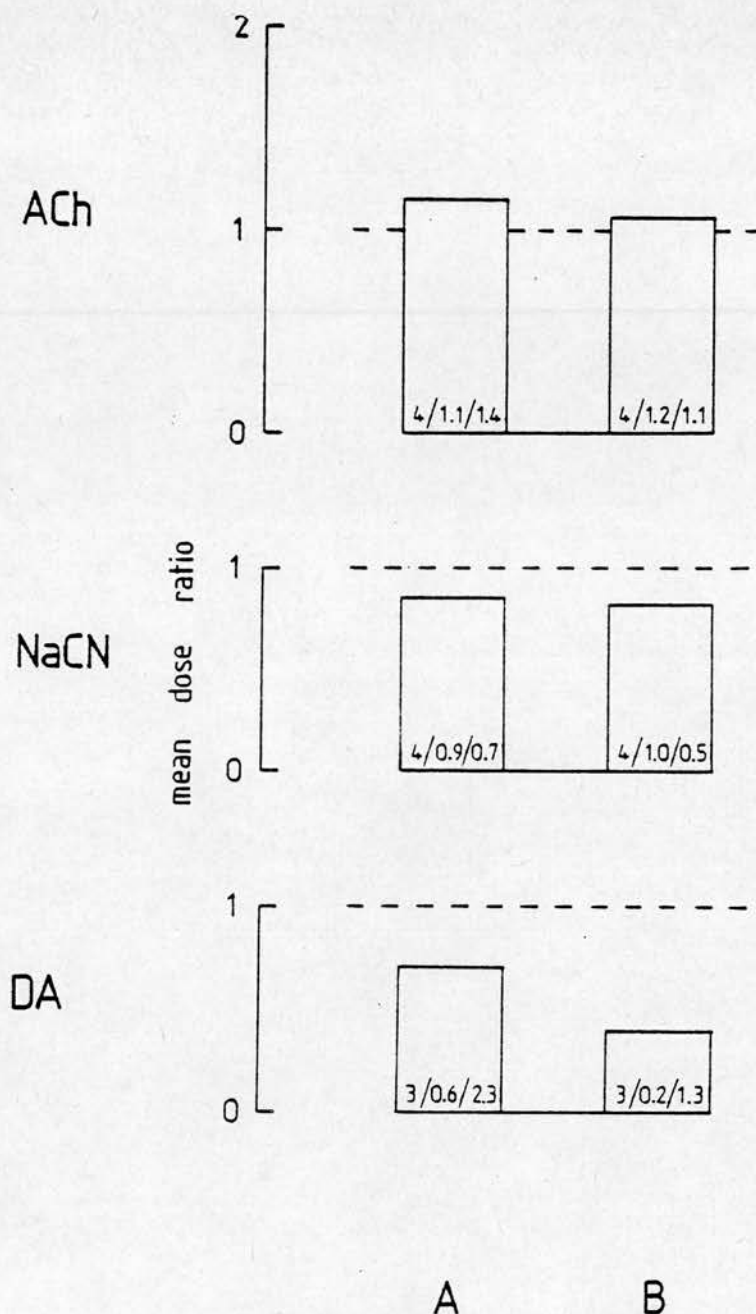


Figure 3.17

Pooled dose-ratio data showing the effect of pargyline, in doses of 2.5 mg /kg I.C. (A) and 5 mg /kg I.C. (B), on chemoreceptor responses to ACh, NaCN and DA. Data are presented as for Fig. 3.5.

an increase in the chemoreceptor response to hypoxia (breathing 10% O<sub>2</sub> in N<sub>2</sub>) following administration of nomifensine (see Figure 3.12B). The frequency of spontaneous discharge in animals breathing room air was also increased (see Table 3.2).

TABLE 3.2: Frequency of spontaneous chemoreceptor discharge in animals breathing room air (arithmetic mean ( $\bar{x}$ )  $\pm$  standard error) before and after administration of nomifensine.

No. of experiments	Dose of nomifensine (mg/kg I.C.)	$\bar{x}$ before	$\bar{x}$ after
3	0.1	2.4 $\pm$ 1.2	6.9 $\pm$ 4.9
2	0.2	1.3 $\pm$ 0.7	2.3 $\pm$ 1.3
1	0.4	2.0	4.2

Mean arterial B.P. was increased by about 25 per cent following administration of 0.1 mg/kg I.C. of nomifensine but was not further modified by an additional dose of 0.2 mg/kg I.C. Mean B.P. fell to control or slightly below control levels following subsequent administration of 0.4 mg/kg I.C.

#### Inhibition of monoamineoxidase (MAO)

##### Pargyline

Pargyline is a potent inhibitor of MAO (see Zeller and Hsu, 1973). The effects of cumulative doses of 2.5 and 5.0 mg/kg I.C. of pargyline on chemoreceptor activity were studied in four experiments. It was found that the inhibitory action of DA was enhanced by pargyline (see Figure 3.16A). Responses to NaCN were, if anything, slightly increased following administration of pargyline while responses to ACh were unaffected or slightly reduced (see Figure 3.15). Dose



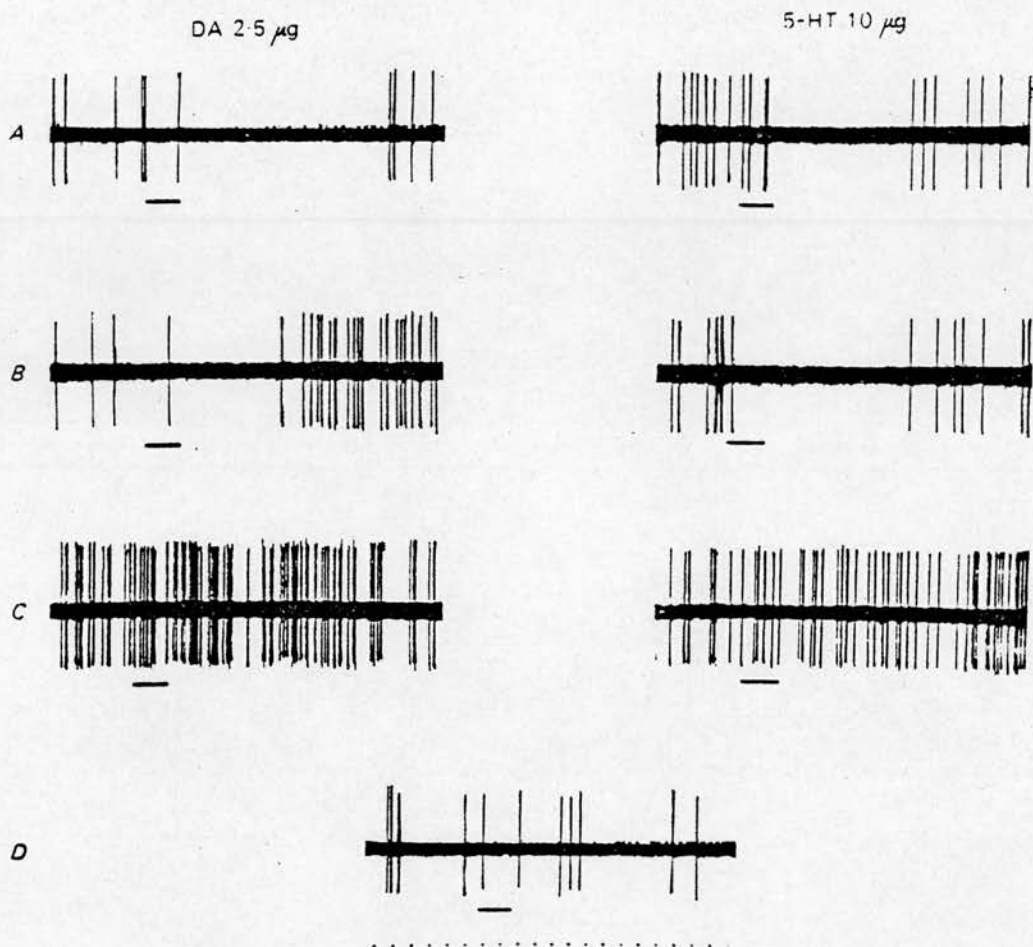


Figure 3.18

Responses of a chemoreceptor unit to DA (left-hand panels) and 5-HT (right hand panels) before (A) and after (B) administration of  $\alpha$ -flupenthixol (0.05 mg /kg I.C.) and after (C) subsequent administration of an additional dose of 0.5 mg /kg I.C. of  $\alpha$ -flupenthixol. Panel D shows the effect of 0.1 ml I.C. of Locke solution (equilibrated with 5% CO<sub>2</sub> in air). Each panel shows, from above downwards : action potentials and injection marker. The one sec timing marks shown below D refer to all five panels.

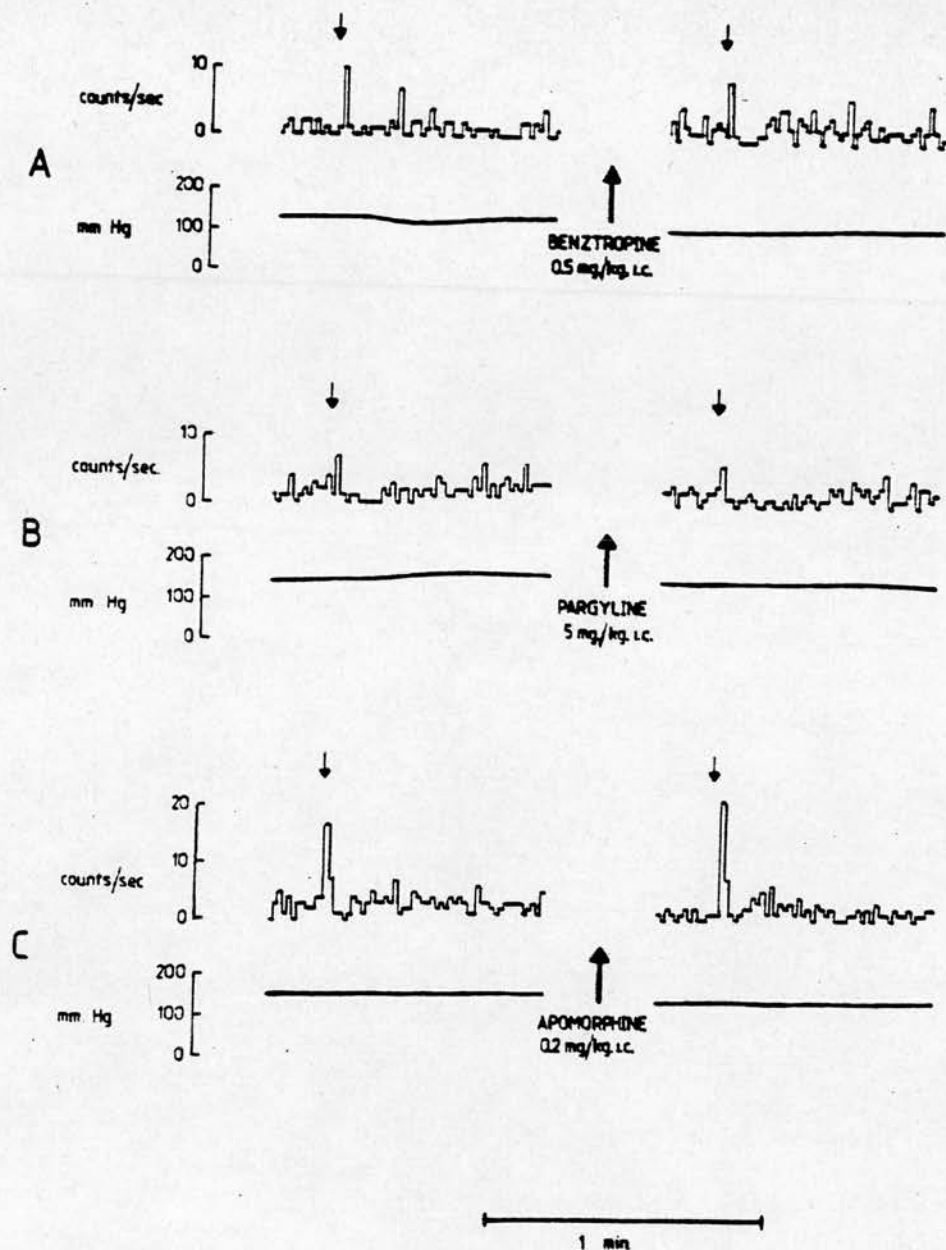


Figure 3.19

Response of carotid chemoreceptors to 5-HT ( $5 \mu\text{g}$  I.C.) before (left-hand panels) and after (right-hand panels) administration of benztropine (A) and pargyline (B) and the response to 5-HT ( $10 \mu\text{g}$  I.C.) before and after administration of apomorphine (C). Recordings were of one, one and two chemoreceptor units in A, B and C respectively. Each panel shows, from above downwards: discharge frequency and mean arterial B.P. Injections of 5-HT were made at the downward pointing arrows. The time bar refers to all six panels.

ratios summarising the effects of pargyline on chemoreceptor responses to DA, NaCN and ACh are shown in Figure 3.17. There was a slight rise in mean arterial B.P. of about 10 per cent following administration of 2.5 mg/kg I.C. of pargyline but no further change was observed after a subsequent dose of 5 mg/kg I.C.

Pargyline had no obvious effect on the chemoreceptor response to hypoxia (see Figure 3.13C) or on the response to hypercapnic Locke solution (0.1 - 0.3 ml I.C.) (see Figure 3.16B). The frequency of spontaneous chemoreceptor discharge in animals breathing room air was  $3.5 \pm 2.4$  counts/sec before and  $8.1 \pm 5.8$  counts/sec after administration of 2.5 mg/kg I.C. of pargyline and  $11.3 \pm 8.0$  counts/sec after an additional dose of 5 mg/kg I.C.

#### Responses to 5-hydroxytryptamine

The effects of single doses (5 or 10  $\mu$ g I.C.) of 5-HT, injected at regular intervals (minimum 5 min) or of a range of doses (0.5 - 5  $\mu$ g I.C.) on chemoreceptor activity were studied in 14 experiments.

5-HT evoked a brief, intense burst of chemoreceptor activity lasting up to 2 sec followed by a period of inhibition lasting up to 10 sec (see Figure 3.18). In three experiments the effect of hypoxia on the response to 5-HT was tested. It was found that the magnitude of the inhibitory response to 5-HT increased when arterial  $pO_2$  was lowered (see Figure 3.3). The magnitude of the initial excitation was unaffected or slightly reduced by hypoxia.

There was a tendency for low doses (< 0.5 mg/kg I.C.) of  $\alpha$ -flupenthixol to reduce the excitatory effect of 5-HT on chemoreceptor activity (see Figure 3.18) although this was not consistent.

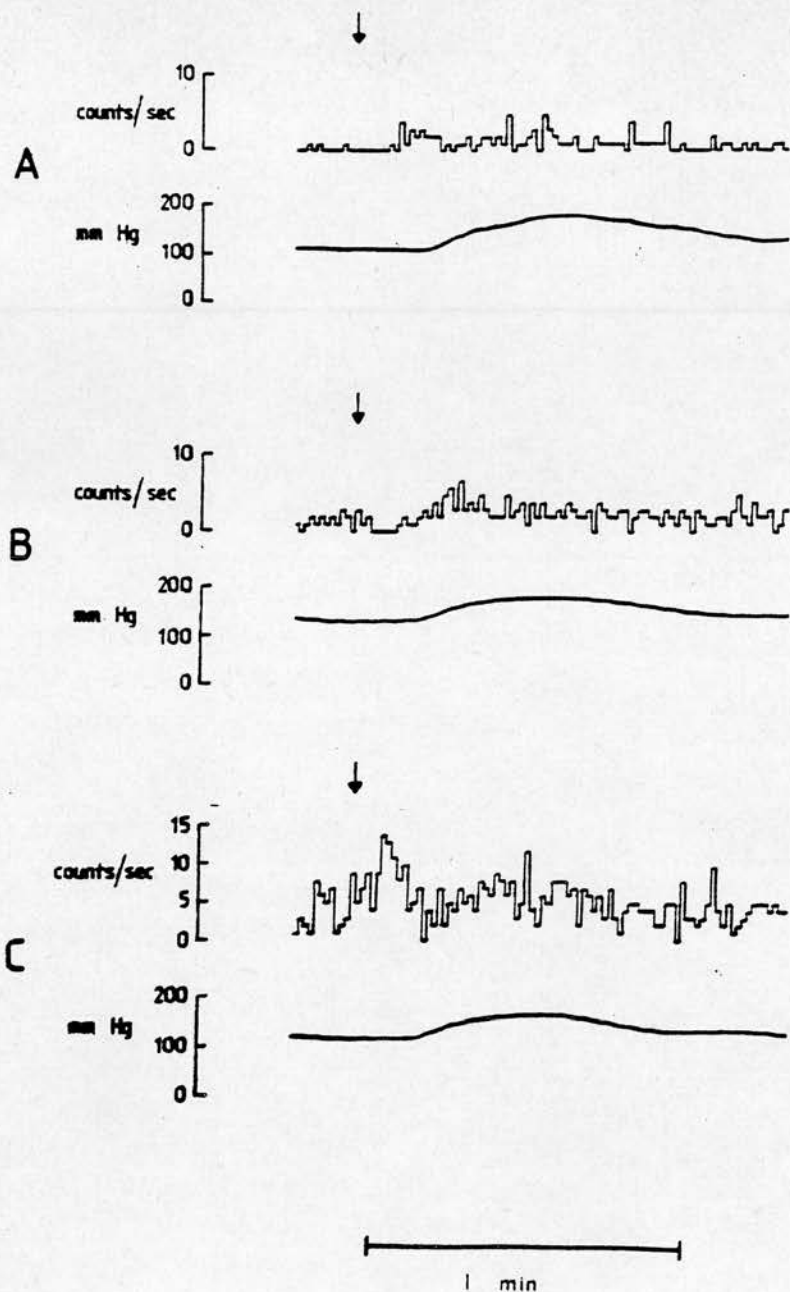


Figure 3.20

Response of carotid chemoreceptors (1 unit) to NA (10 µg I.C.) before (A) and after (B) administration of nomifensine (0.2 mg/kg I.C.) and after (C) subsequent administration of  $\alpha$ -flupenthixol (0.2 mg/kg I.C.). Each panel shows, from above downwards: discharge frequency and mean arterial B.P. Injections of NA were made at the arrows. The time bar refers to all three panels.

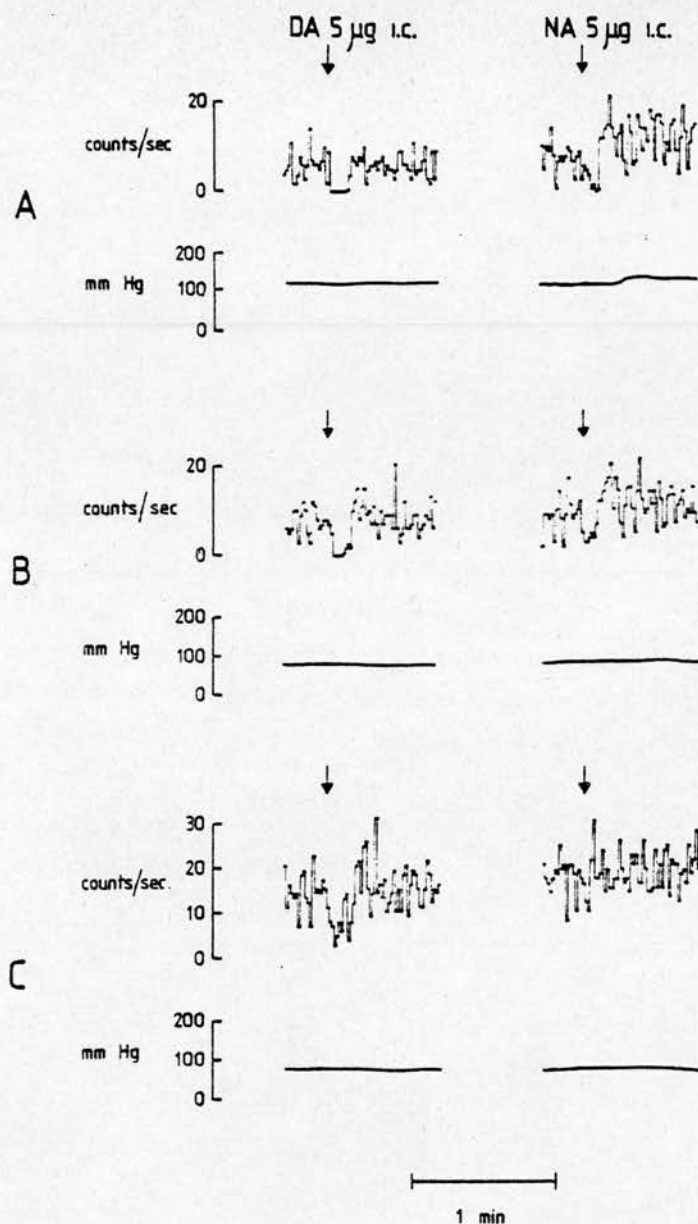
The inhibitory effect of 5-HT was not appreciably affected until high doses of  $\alpha$ -flupenthixol ( $\geq 0.5$  mg/kg I.C.) were administered, when the response was abolished (see Figure 3.18). This finding meant that results obtained following high doses of  $\alpha$ -flupenthixol had to be interpreted cautiously because the drug was evidently no longer acting selectively. The chemoreceptor response to 5-HT was not, however, appreciably affected by benztropine or pargyline (see Figures 3.19A, 3.19B) or when spontaneous discharge was depressed by apomorphine (see Figure 3.19C).

#### Responses to noradrenaline

Chemoreceptor responses to single doses (5 or 10  $\mu$ g I.C.) or a range of doses (2.5 - 25  $\mu$ g I.C.) of NA were obtained in seven experiments. NA, in the doses studied, evoked an immediate, short-lasting (1 - 20 sec) inhibition of chemoreceptor activity followed by a secondary excitation (see Figures 3.14C, 3.20, 3.21). The initial inhibitory effect of NA was less marked than that produced by DA and was less consistent, being observed in response to only 13 of a total of 21 injections. The secondary excitation had a similar time course to the pressor response evoked by NA and it is tempting to speculate that such excitation may be due to a reduction in carotid body blood flow as a consequence of the vasoconstrictor action of the drug.

Nomifensine (0.1 - 0.4 mg/kg I.C.) enhanced both the initial inhibition, when such was observed, and the secondary excitation evoked by NA, and, on some occasions, potentiated the pressor response (see Figures 3.14C, 3.20). Administration of  $\alpha$ -flupenthixol (0.2 mg/kg I.C.) subsequent to nomifensine (consecutive doses of





**Figure 3.21**

Response of carotid chemoreceptors (2 units) to DA (left-hand panels) and NA (right-hand panels) before (A) and after (B) administration of PBA (5 mg/kg I.V.) and after (C) an additional dose of PBA (5 mg/kg I.V.). Each panel shows, from above downwards: discharge frequency and mean arterial B.P. Injections of DA and NA were made at the arrows. The time bar refers to all six panels.

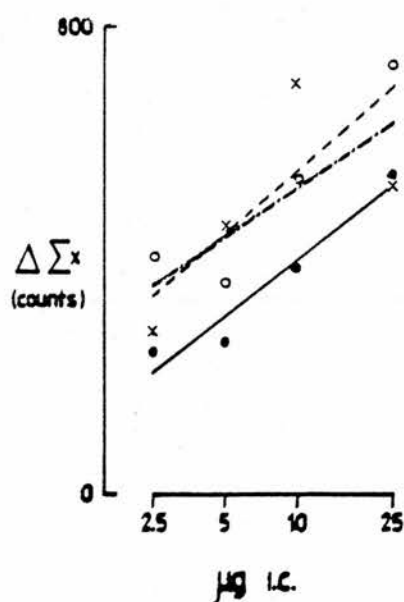


Figure 3.22

Dose-response data for NaCN, from a recording of two chemoreceptor units, before (●—●) and after (○--○) administration of PBA (5 + 5 mg /kg I.V.) and after (x--x) subsequent administration of propranolol (2 mg /kg I.V.). Doses are plotted on a  $\log_{10}$  scale and lines fitted by the method of least squares.

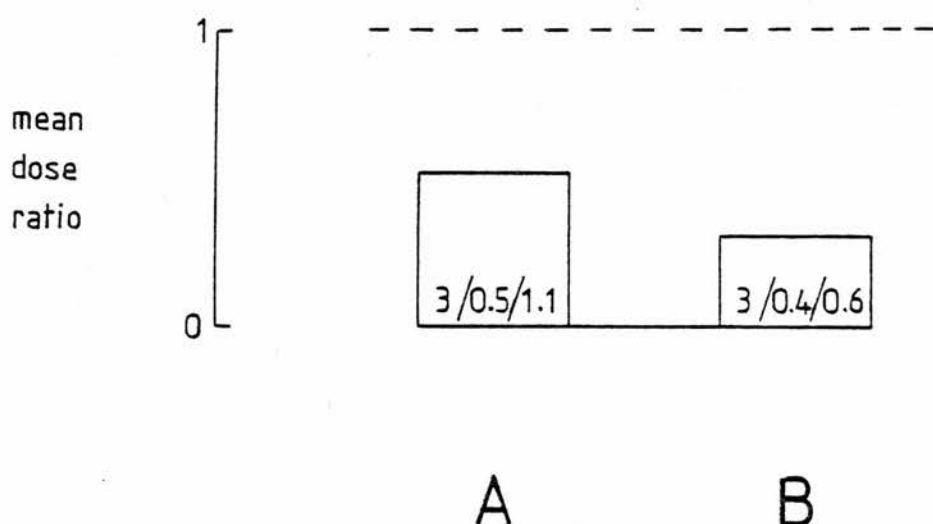


Figure 3.23

Pooled dose-ratio data showing the effect of PBA, in doses of 5 mg /kg I.V. (A) and 5 + 5 mg /kg I.V. (B), on chemoreceptor responses to NaCN. The above data were obtained from three recordings of chemoreceptor activity in two experiments. Data are presented as for Fig. 3.5.

0.1 and 0.2 mg/kg I.C.) blocked the inhibitory, but not the excitatory, effects of NA (see Figure 3.20). This dose of  $\alpha$ -flupenthixol had little effect on the pressor response to NA (see Figure 3.20).

#### Effect of phenoxybenzamine (PBA)

Two experiments were performed in which the effects of the  $\alpha$ -adrenergic blocking agent PBA on chemoreceptor activity were investigated. The effects of an initial dose of 5 mg/kg I.V. of PBA and a subsequent dose, also of 5 mg/kg I.V., were studied in both experiments. A period of 30 min was allowed following administration of PBA before responses to test substances were obtained. Mean arterial B.P. fell by 20 - 30 per cent following administration of the initial dose but was not further reduced by the second dose.

The chemoreceptor response to NaCN was enhanced following administration of PBA. This effect was not reversed by administration of the  $\beta$ -adrenergic blocking agent propranolol (2 mg/kg I.C.) (see Figure 3.22). Dose-ratios summarising the effect of PBA on responses to NaCN are shown in Figure 3.23.

Inhibitory responses to NA and DA were little affected following administration of the initial dose of PBA although the secondary excitatory effects of NA were reduced. The inhibitory response to NA was reduced, however, following administration of the second dose of PBA (see Figure 3.21). DA still evoked an inhibition of chemoreceptor activity following administration of the second dose of PBA but the response became inconsistent (see Figure 3.21). The pressor response to NA was abolished by PBA (see Figure 3.21).

Spontaneous chemoreceptor discharge frequency in animals breathing room air was  $5.0 \pm 1.2$  counts/sec before and  $6.1 \pm 1.9$  counts/sec after an initial dose of 5 mg/kg I.V. of PBA and  $11.0 \pm 2.3$  counts/sec after an additional dose of 5 mg/kg I.V.

### DISCUSSION

The present results confirm that I.C. injection of low doses of DA inhibits chemoreceptor activity in the cat carotid body *in situ* (see also Black *et al*, 1976a; Nishi, 1977; Zapata, 1977; Llados and Zapata, 1978a; Zapata and Llados, 1978). It is unlikely that the inhibitory effect is a consequence of a change in carotid body blood flow since DA is also found to inhibit chemoreceptor activity in superfused preparations *in vitro* (Zapata, 1975) and during stagnant asphyxia in the carotid body *in situ* (Sampson *et al*, 1976a). It appears that DA inhibits chemoreceptor activity by a direct action on sensory nerve endings or on structures presynaptic to sensory nerve endings in the carotid body.

The magnitude of the inhibitory response to DA was found to be inversely proportional to arterial  $pO_2$ , i.e. proportional to the background frequency of chemoreceptor discharge against which the inhibition was measured. A similar result was obtained by Nishi (1977). Sampson *et al* (1976a) found that there was no difference in the chemoreceptor response to DA in animals breathing room air or animals ventilated with hypoxic gas mixtures. The latter authors, however, expressed their results as percentage changes in average discharge frequency, whereas in the present study the absolute difference in discharge,  $\Delta\Sigma x$ , was measured (see Section II). Since the magnitude of an inhibitory response, measured as  $\Delta\Sigma x$ ,

is proportional to the frequency of spontaneous discharge, this meant that the effects of drugs which change the background frequency of spontaneous chemoreceptor discharge on responses to DA had to be interpreted cautiously.

High doses ( $\geq 10 \mu\text{g}$  I.C.) and occasionally low doses ( $< 10 \mu\text{g}$  I.C.) of DA caused a delayed increase in discharge which reduced the duration of the inhibitory response. Sampson (1972) and Sampson *et al* (1976a, 1976b) obtained repeatable inhibitory responses to DA *in situ* using doses of 2 - 5  $\mu\text{g}$  I.C. which accords with the present results at these doses. They found, however, that DA produced "only a depression of impulse activity" (Sampson *et al*, 1976a) whereas secondary excitations were observed, albeit infrequently, even with low doses of DA in the present study. Llados and Zapata (1976a) found that low doses of DA ( $\leq 5 \mu\text{g}$  I.C.) caused inhibition of chemoreceptor activity and that on "certain occasions" this inhibitory effect was followed by a secondary excitation which is in complete agreement with the present results. Nishi (1977) found that injection of small doses of DA (0.2 - 0.5  $\mu\text{g}$  I.C.) evoked an initial brief increase in chemoreceptor activity which preceded the inhibitory effect. This phenomenon was not observed in the present study. The above authors did not study the effects of higher doses of DA which were found in the present investigation to give delayed increases in discharge and inconsistent inhibitory responses. The time course of the delayed increase in discharge was similar to that of the slight pressor response observed following administration of high doses of DA which suggests that the two effects might be related. This delayed increase in discharge, however, seems similar to that observed by Zapata (1975) *in vitro*



and it may be premature, therefore, to attribute the excitatory effect observed *in situ* to circulatory changes. This view is supported by the finding that the delayed excitatory effect produced by DA *in situ* persists, even after the pressor response to DA is blocked by administration of dibenamine (Llados and Zapata, 1978a). The present investigation, however, was concerned mainly with the inhibitory effect of DA and the mechanism of the delayed excitatory effect was not studied.

Following administration of  $\alpha$ -flupenthixol (0.2 - 1.0 mg/kg I.C.) the inhibitory response of the carotid chemoreceptors to DA was abolished and an excitatory response was observed. Similar results were obtained with another DA antagonist, haloperidol. These results are in agreement with those obtained by other workers who used haloperidol and trifluoperidol (Nishi, 1977) or haloperidol, spiroperidol, fluphenazine and chlorpromazine (Llados and Zapata, 1978a) to block the DA inhibitory response.

In the present experiments, administration of DA blockers had little effect on spontaneous discharge frequency in animals breathing room air. Zapata (1975) found that spiroperidol had little effect on spontaneous chemoreceptor discharge frequency *in vitro*. Nishi (1977) and Llados and Zapata (1978a), however, found that administration of DA blockers caused a marked increase in discharge frequency *in situ*. This discrepancy in results is difficult to explain since the experimental method used by these workers was very similar to that used in the present study save that in the present experiments gallamine was used to paralyse spontaneous respiration. It is possible that gallamine prevented an increase in discharge following administration of DA blockers although previous work has

shown that gallamine itself, in the doses used in the present study, has little effect on chemoreceptor activity in the cat carotid body (McQueen, 1977). A slight increase in the frequency of spontaneous discharge was observed following administration of a high dose of  $\alpha$ -flupenthixol (1 mg/kg I.C.) in the present experiments but at this dose the inhibitory effect of 5-HT was also blocked, indicating that the drug was no longer acting selectively against DA, which it was assumed to be at lower doses.

If the theories of Osborne and Butler (1975) or Krammer (1978) are correct (see Section I) and DA is released from type I cells to suppress activity in otherwise spontaneously active sensory nerve ends then abolition of the inhibitory effect of DA would be expected to precipitate a marked increase in chemoreceptor activity. While the results obtained by Nishi (1977) and by Llados and Zapata (1978a), discussed above, may be interpreted in favour of such an hypothesis, the results obtained in the present study and by Zapata (1975) are not in accord with such an idea. Osborne and Butler also suggest that a transmitter, possibly ACh, is released from sensory nerve ends and acts to suppress the release of DA from type I cells, thereby causing an increase in chemoreceptor discharge. If this is the case then ACh should no longer cause excitation when the inhibitory effect of DA is blocked. It was found in the present study that the response to ACh was little affected by doses of  $\alpha$ -flupenthixol sufficient to block the inhibitory effects of DA. High doses of  $\alpha$ -flupenthixol ( $\geq 0.5$  mg/kg I.C.) did reduce the response to ACh but, at these doses, it was not certain whether  $\alpha$ -flupenthixol was acting selectively against DA (see above). Similar results were obtained with haloperidol. Nishi (1977) found that the chemoreceptor

response to ACh was unchanged or enhanced by haloperidol. These results do not support the theory advanced by Osborne and Butler.

As well as blocking the inhibitory response to DA,  $\alpha$ -flupenthixol potentiated chemoreceptor response to NaCN and increased the rate of increase of chemoreceptor discharge during hypoxia. Similar results were obtained using haloperidol and Nishi (1977) has also reported that haloperidol causes potentiation of responses to chemoreceptor stimuli. These results are compatible with suggestions that endogenous DA may, through an inhibitory action, modulate chemosensory activity (McDonald and Mitchell, 1975; Zapata, 1975). This conclusion relies on the assumption that potentiation of responses to chemoreceptor stimuli was a consequence of blockade of an inhibitory effect of endogenous DA. Such an assumption is not unwarranted, however, since DA is stored in the carotid body and released during hypoxia (see Section I) and DA can inhibit chemoreceptor activity. The chemoreceptor response to ACh was not, however, appreciably affected by  $\alpha$ -flupenthixol or haloperidol. It may be that ACh increases afferent chemoreceptor activity by a direct action on the unmyelinated, terminal portion of the sensory nerves, as Douglas (1954) suggested, and that the dopaminergic mechanism operates at a point which is peripheral to this site of action, e.g. at synapses between type I cells and sensory nerve endings. If this is the case then drugs which modify the action of endogenous DA would not be expected to have much effect on responses to ACh.

In addition to studying the effects of DA-blocking drugs, experiments were performed to study the activity of the carotid chemoreceptors during prolonged stimulation of the DA inhibitory receptor. Inhibitory responses to DA injections were short-lasting,

and although the use of DA infusion to give a sustained inhibition was considered, this was rejected because of the possibility of causing delayed increases in discharge. Instead, the DA agonist apomorphine was used and gave a long-lasting inhibition of chemoreceptor activity without any apparent excitatory effects. Nishi (1977) and Llados and Zapata (1978a) obtained a similar result with apomorphine. Responses to NaCN and ACh were slightly reduced by apomorphine and the response to hypoxia was depressed. Nishi (1977) found that responses to both NaCN and hypoxia were reduced by administration of doses of apomorphine similar to those used in the present study although responses to the nicotinic stimulant DMPP were not. It seems then that chemoreceptor responses to hypoxia and NaCN may be reduced by apomorphine. If the depressant effects of apomorphine are due to an action on the DA inhibitory receptor then these results support the idea that endogenous DA has an inhibitory effect on chemoreceptor activity. The inhibitory effects of apomorphine were prevented by administration of  $\alpha$ -flupenthixol, an observation which provides indirect evidence that DA and apomorphine were acting at the same site(s).

As an alternative to studying the effects of injections of DA or apomorphine, it was decided to study the effects of amphetamine in the hope that this drug, by releasing DA from endogenous stores, would give a better indication of the action of endogenous DA. If endogenous DA acts as an inhibitory modulator of chemoreceptor activity, as the above results suggest, then one might expect amphetamine to inhibit chemoreceptor activity. Low doses of amphetamine (5 - 50  $\mu$ g I.C.) did inhibit chemoreceptor activity but the response was inconsistent and did not appear to be dose-related,



while a high dose of amphetamine (0.25 mg/kg I.C.) caused stimulation of activity. These results are puzzling if endogenous DA is an inhibitory modulator of chemoreceptor activity. It is possible that DA stores in the carotid body are resistant to the DA-releasing action of amphetamine. If this is the case then the inhibitory effect of low doses of amphetamine might have been due to a slight agonist action of amphetamine on DA inhibitory receptors. The stimulant effect of the high dose of amphetamine is difficult to explain however. Llados and Zapata (1978a) found that doses of amphetamine up to 2 mg I.C. "did not induce inhibition" of chemoreceptor activity but do not mention whether or not they observed any stimulant effects. It may be that the stimulant effect of amphetamine observed in the present study was unrelated to the DA-releasing action of the drug. Nevertheless, since DA may cause excitation of chemoreceptor activity as well as inhibition (see above), the possibility that the stimulant effect of amphetamine was a consequence of release of endogenous DA cannot be ruled out.

If DA acts as a modulator of afferent chemoreceptor activity then the most likely place for such a mechanism to operate is at synapses between sensory nerve endings and type I cells. Ultrastructural studies of the innervation of the carotid body have shown that type I cells are in synaptic contact with nerve endings (see Biscoe, 1971). There is, however, a dispute as to whether the nerves forming these synaptic connections are sensory as claimed by De Castro (1928) or whether they are part of an efferent pathway (Biscoe, Lall and Sampson, 1969). Current evidence suggests that the majority of the fibres are sensory (Hess and Zapata, 1972; Nishi and Stensaas, 1974; Osborne and Butler, 1975; Mitchell and McDonald,



1975; Kondo, 1976) although the synaptic connections between the sensory nerves and type I cells may be reciprocal (Verna, 1973; Butler and Osborne, 1975; Mitchell and McDonald, 1975). If DA is released from type I cells at these synapses and acts on sensory nerve ends then it is likely that there is a mechanism for inactivation of released transmitter. This could be accomplished by enzymatic degradation, by cellular uptake, by diffusion of DA away from the synapse or by a combination of these processes. Lee and Mattenheimer (1965) demonstrated the presence of MAO in the carotid body of the bullock. Since DA is a substrate for MAO it is possible that this enzyme is involved in the inactivation mechanism for DA released from type I cells. It was found, however, that pargyline, a MAO inhibitor, had little effect on chemoreceptor responses to NaCN, ACh, hypoxia or hypercapnic Locke solution. Inhibitory responses to DA were enhanced following administration of pargyline although this may, in part, have been due to the increased background discharge frequency against which the inhibition was measured. If endogenous DA modifies responses to chemoreceptor stimuli as the results from experiments with DA receptor blocking agents suggest (at least for NaCN and hypoxia), then the results from experiments with pargyline suggest that MAO may contribute, but is not very important to the inactivation mechanism for DA in the region of the synapse at which the presumptive dopaminergic mechanism operates. The increased response to injected DA may have been due in part, to blockade of MAO at a site which is remote from the synapse between sensory nerve ends and type I cells. It is possible that another enzyme or enzymes, such as catechol-O-methyltransferase (COMT) is of importance in the inactivation of DA in the synaptic

region. So far as I am aware, however, the distribution of COMT in the carotid body or the effects of COMT inhibitors on chemoreceptor activity have not been studied.

Cellular uptake is an important mechanism for the inactivation of released neurotransmitter and for control of release in adrenergic nerves and also in dopaminergic neurons in the C.N.S. (see Iversen, 1974). Type I cells in the carotid body are not neurons, however, and Mills *et al* (1978) have suggested that these cells may have no mechanism for catecholamine uptake. Kobayashi's (1975) autoradiographic studies, however, showed that DA was taken up into some, though not all, type I cells in the mouse carotid body and Lishajko (1970) has demonstrated uptake of tritiated DA into isolated granules of human carotid body tumour. If cellular uptake is important as an inactivation mechanism for DA released during chemoreceptor stimulation then drugs which block DA uptake might be expected to enhance the effects of endogenously released DA. Alternatively if DA uptake is not important then one would not expect drugs which block DA uptake to have any appreciable effect on chemoreceptor activity, unless by a non-specific action. Hence, if DA acts as an inhibitory modulator of chemoreceptor activity, drugs which block DA uptake would be expected to depress responses to chemoreceptor stimuli or to have no effect, depending on whether or not uptake was an important inactivation mechanism. In contrast to expected results, however, benztropine and nomifensine, both potent inhibitors of DA uptake, potentiated chemoreceptor responses to NaCN and hypoxia. Benztropine also potentiated the response to hypercapnic Locke solution. If these effects were due to blockade of DA uptake at the synapse between type I cells and sensory nerve endings, then the

results suggest that endogenous DA has an excitatory effect on chemoreceptor activity. That both benztropine and nomifensine blocked DA uptake is evidenced by the observation that both caused a marked potentiation of the inhibitory response to injected DA. Chemo-receptor responses to ACh were unaffected by either nomifensine or low doses of benztropine. It may be, however, as suggested above, that ACh stimulates afferent chemoreceptor activity by a direct action on the terminal, unmyelinated portion of the sensory nerve and so acts at a point which is central to that at which a dopaminergic mechanism operates. The higher dose of benztropine reduced responses to ACh but this is not surprising since benztropine, as well as blocking DA uptake, has anticholinergic properties.

The above suggestion that endogenous DA may have an excitatory effect on chemoreceptor activity does not invalidate the earlier suggestion that endogenous DA has an inhibitory effect. It may be that endogenous DA has both an inhibitory and an excitatory effect on chemoreceptor activity. Zapata (1977) proposed such a dual role for endogenous DA and Lladós and Zapata (1978a) have suggested that even in a preparation in which the response to injected DA is inhibitory, "excitation could result in response to liberation of endogenous DA ... if there is different accessibility to the two types of DA receptors", i.e. excitatory and inhibitory DA receptors. It may be that there are both excitatory and inhibitory receptors for DA in the carotid body and that the juxtaposition of the receptors on the sensory nerve end is such that the inhibitory receptors are freely accessible to injected DA and the excitatory receptors are less so, while the excitatory receptors are more accessible than the inhibitory receptors to endogenous DA. Such a

situation might occur if the excitatory receptors are located on the sensory nerve end, mainly in the region where this is apposed to a type I cell, i.e. in the synaptic cleft, and the inhibitory receptors are located on the periphery of the synaptic region and on the extrasynaptic region of the sensory nerve end. Under normal conditions DA release would be minimal and affect mainly the excitatory receptors - hence blockade of the inhibitory receptors with  $\alpha$ -flupenthixol has little effect on resting chemoreceptor activity. When the chemoreceptors are stimulated, DA release increases and the number of excitatory receptors activated increases but the number of inhibitory receptors activated increases also. Blocking the inhibitory receptors under these conditions would tend to increase chemoreceptor activity and hence  $\alpha$ -flupenthixol potentiates responses to chemoreceptor stimuli. DA uptake blockers increase the amount of DA acting at both inhibitory and excitatory receptors but since excitatory receptors predominate at the site of DA release, chemoreceptor responses to stimulants are potentiated. Administration of  $\alpha$ -flupenthixol subsequent to benztropine caused a marked potentiation of responses to NaCN which would be expected if the above were the case. This interpretation also explains why high doses of DA caused a delayed increase in discharge and even low doses stimulate chemoreceptor discharge following administration of  $\alpha$ -flupenthixol. In addition this could also explain why high doses of amphetamine caused a stimulation of chemoreceptor discharge.

Kebabian and Calne (1979) have suggested that at least two distinct types of DA receptor, designated D1 and D2, are present in the mammalian C.N.S. The possibility that the excitatory and inhibitory DA receptors in the carotid body correspond to the central



D1 and D2 receptors proposed by these authors is discussed further in Section V.

From a consideration of the above, a 'self-modulating' mechanism for the action of endogenous DA is proposed, i.e. DA is released from type I cells and acts on excitatory receptors on the sensory nerve end but the excitatory effect of DA on chemoreceptor activity is limited by the presence of inhibitory receptors which are less accessible to endogenous DA than the excitatory receptors but which are activated when the amount of DA released has reached a sufficient level. This conclusion, however, relies on the assumption that DA uptake blockers potentiated responses to chemoreceptor stimuli by blocking cellular uptake of DA at the synapse between type I cells and sensory nerve endings and also that drugs which blocked the inhibitory effect of injected DA potentiated responses to chemoreceptor stimuli by blocking an inhibitory action of endogenous DA. It may be worthwhile considering other possible interpretations of results from experiments with drugs which block the DA inhibitory receptor or prevent uptake. It is possible, for example, that the effects of these drugs were a consequence of changes in carotid body blood flow or an action on a noradrenergic or serotonergic mechanism since the carotid body contains both NA and 5-HT (see Section I).

#### Blood flow

An increase in carotid body blood flow would be expected to decrease, and a reduced flow to increase, the frequency of spontaneous chemoreceptor discharge in animals breathing room air (see Torrance, 1968). Neither  $\alpha$ -flupenthixol nor haloperidol had any appreciable effects on resting discharge frequency. It is



unlikely that the effects of these drugs are attributable to flow changes. Both benztropine and nomifensine, however, increased resting discharge frequency and it is possible therefore that these drugs reduced carotid body blood flow. If this occurred, it is unlikely to have resulted from an effect on the general circulation since benztropine caused a slight decrease, and nomifensine (0.1 and 0.2 mg/kg I.C.) caused a slight increase, in arterial B.P. The drugs may, however, have had a particular effect on carotid body blood flow. If carotid body flow was reduced, however, the dose of stimulant drugs reaching the chemoreceptors would also be reduced since a smaller amount of carotid blood would enter the carotid body and drugs were injected into the carotid artery. Nevertheless, if decreased flow reduced  $O_2$  availability to the chemoreceptors (but see Torrance, 1968) responses to NaCN and  $CO_2$  could still be potentiated since the effects of both stimulants are interactive with hypoxia, i.e. the chemoreceptor response is greater when  $O_2$  levels are reduced (Lahiri, 1977). This argument does not apply to ACh, however, since the chemoreceptor response to ACh is additive with hypoxia, i.e. the magnitude of the response to ACh is not dependent on  $O_2$  levels (Lahiri, 1977). Since chemoreceptor responses to ACh were not appreciably affected by nomifensine or by low doses of benztropine it is unlikely that the dose of ACh reaching the chemoreceptors was changed. Hence, blood flow through the carotid body was probably little affected by the DA uptake blockers. An explanation of the effects of benztropine and nomifensine based on flow changes is, therefore, untenable.

## NA

$\alpha$ -Flupenthixol, though more selective for DA receptors, may also block  $\alpha$ -adrenergic receptors (Moller Nielsen *et al*, 1973) and benztropine and nomifensine can block NA uptake (Horn, Coyle and Snyder, 1971; Hoffmann, 1977). It is possible, therefore, that the observed effects of these drugs are a consequence of their action on a noradrenergic rather than a dopaminergic mechanism.

I.C. injection of NA caused a brief inhibition of chemoreceptor activity followed by excitation. This result is in good agreement with results obtained by other workers (Sampson, 1972; Sampson *et al*, 1976a, 1976b; Llados and Zapata, 1978b; see also Section I). The time course of the delayed excitatory effect was similar to the time course of the pressor response to NA and was probably due to reduced blood flow through the carotid body as a consequence of vascular changes (see Section I). Llados and Zapata (1978b) found that I.C. injection of NA did not always produce an inhibitory response and attributed this effect, when observed, to a 'flush response' or to a weak action of NA on DA inhibitory receptors. Great care was taken in the present experiments to avoid 'flush responses' (see Section II) and it is unlikely that this phenomenon accounts for the inhibitory effect of NA observed in these experiments. In agreement with Llados and Zapata, however, it was found that NA did not always produce an inhibitory effect.

The inhibitory response to NA was prevented by administration of a high dose of the  $\alpha$ -adrenergic blocking agent PBA (5 + 5 mg/kg I.V.) but not by a lower dose (5 mg/kg I.V.) although the pressor response was blocked by either dose. Also, the lower dose of PBA had little effect on resting chemoreceptor discharge frequency but

the higher dose caused an increase in frequency. Similar results were obtained by Lladós and Zapata (1978b) who suggested that blockade of the inhibitory response to NA was an indirect effect resulting from the hypotension produced by high doses of PBA. It was found in the present experiments, however, that although administration of the lower dose of PBA caused a 20 - 30 per cent reduction in B.P., the inhibitory response to NA was not affected while increasing the dose of PBA blocked the inhibitory response to NA without further modifying B.P. It seems unlikely, therefore, that blockade of the NA inhibitory response by the higher dose of PBA was due to the development of hypotension.

Chemoreceptor responses to NaCN were potentiated following administration of PBA. Since high doses of PBA blocked the inhibitory response of the chemoreceptors to NA and since  $\alpha$ -flupenthixol also blocked this effect, it is possible that potentiation of chemoreceptor responses to NaCN following administration of  $\alpha$ -flupenthixol was a consequence of blockade of  $\alpha$ -adrenergic receptors rather than DA receptors and that this is also the mechanism for the potentiating effect of PBA. These results then, might be interpreted in favour of the idea that  $\alpha$ -adrenergic receptors mediate inhibition of chemoreceptor activity *in vivo* (see Section I). If this is the case, however, it is difficult to explain the effects of benztropine and nomifensine on the chemoreceptor response to NaCN. Nomifensine potentiated the chemoreceptor response to injected NA. It is possible that nomifensine and benztropine block cellular uptake of NA, released during chemoreceptor stimulation, and that endogenous NA stimulates afferent chemoreceptor activity by a  $\beta$ -adrenergic action. This is unlikely, however, since propranolol, a  $\beta$ -adrenergic

antagonist, did not reverse the potentiation of responses to NaCN caused by PBA and  $\beta$ -adrenergic blockers do not otherwise have any appreciable effect on chemoreceptor activity (Llados and Zapata, 1978b; see also Section I). In any case, it is unlikely that the inhibitory effect of NA is mediated by  $\alpha$ -adrenergic receptors since the low dose of PBA which blocked the pressor response to NA, had little effect on NA-induced inhibition of chemoreceptor activity.

From the above it may be seen that it is difficult to explain the effects of DA antagonists or uptake blockers in terms of any non-specific effects they may have on a noradrenergic mechanism. It is possible, however, that the effects of NA and PBA were a consequence of non-specific effects of these substances on a dopaminergic mechanism. It may be, as Llados and Zapata suggest (see above), that the inhibitory effect of NA is due to a weak agonist action of NA on DA inhibitory receptors. Also, PBA has been shown to enhance the release of DA from rat brain slices during electrical stimulation (Howd and Horita, 1975) and it is possible that the drug may also increase the release of DA from type I cells in the carotid body during chemoreceptor stimulation which, if endogenous DA stimulates chemoreceptor activity, would be expected to cause potentiation of responses to chemoreceptor stimuli. The lack of drugs which act specifically on either noradrenergic or dopaminergic mechanisms make this a difficult problem to study. An indication of the effects, if any, of endogenous NA on chemoreceptor activity might be obtained by studying the effects of the catecholamine uptake blocker desipramine, which is about one thousand times more potent for blocking NA than DA uptake (Foster,

1967), on chemoreceptor activity. This experiment, however, was not performed in the present study.

### 5-HT

The effects of 5-HT and of some antagonists of 5-HT on chemoreceptor activity in the cat carotid body were the subject of a recent study by Nishi (1975) (see Section I). The results obtained with 5-HT in the present study were similar to those obtained by Nishi. The excitatory effect of 5-HT was blocked by low doses of  $\alpha$ -flupenthixol but the inhibitory response was not affected until high doses of  $\alpha$ -flupenthixol ( $\geq 0.5$  mg/kg I.C.) were administered. This result is not surprising since  $\alpha$ -flupenthixol, although a potent DA antagonist, also antagonizes the effects of 5-HT in the C.N.S., being only slightly more selective for DA (Straughan and Dray, 1976). The chemoreceptor response to 5-HT was not appreciably affected by benztropine however, and it is unlikely that the effects of DA antagonists or uptake blockers on chemoreceptor activity were due to a non-specific effect on a serotonergic mechanism. Surprisingly, responses to 5-HT were unaffected by pargyline although 5-HT is a good substrate for MAO. This is further evidence that MAO activity in the cat carotid body is low. Starlinger (1977) attributed this low MAO activity in the cat carotid body to the presence of an endogenous inhibitor of the enzyme.

### Summary

From a consideration of the above it can be seen that the effects of DA antagonists or uptake blockers on chemoreceptor activity cannot be satisfactorily explained in terms of changes in carotid body blood flow or any non-specific actions they may have on



a serotonergic mechanism. It is possible that potentiation of responses to chemoreceptor stimuli following administration of  $\alpha$ -flupenthixol is due to blockade of  $\alpha$ -adrenergic receptors although it seems more likely that this is due to blockade of DA inhibitory receptors and, in any case, the potentiating effect of DA uptake blockers on responses to chemoreceptor stimuli is difficult to explain in terms of a noradrenergic mechanism. Further experiments are required to elucidate the role, if any, of NA in the carotid chemoreceptors. The most satisfactory explanation of the results is that DA has both excitatory and inhibitory effects on carotid chemoreceptor activity mediated by at least two distinct DA receptors in the manner described above. This does not mean to say that the type I cells in the carotid body are the chemoreceptors and that DA-induced increase of chemoreceptor activity is the sole mechanism for stimulation of afferent chemoreceptor activity, although this is a possibility.

#### SECTION IV

#### THE EFFECTS OF ACETYLCHOLINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE ON CHEMORECEPTOR ACTIVITY IN THE RABBIT CAROTID BODY

## INTRODUCTION

The present electrophysiological investigation was undertaken to examine the effects of ACh on carotid chemosensory activity in the rabbit.

ACh has been shown to stimulate carotid chemoreceptor activity in the cat and dog and it has been suggested that this substance may act as a sensory transmitter in the carotid body (see Section 1). It was therefore of interest to determine the effects of ACh on carotid chemosensory activity in the rabbit since at the time the present investigation was instituted, there were no reports available in the literature concerning the effects of ACh or of drugs which affect the cholinergic system on chemoreceptor activity in this species.

The opportunity was taken to investigate the effects of DA and 5-HT on the activity of rabbit carotid chemoreceptors since reports of the effects of these substances on chemoreceptor activity in the cat and dog suggest that their effects are species-specific (see Section 1).

## RESULTS

Experiments were performed on 86 rabbits (six female New Zealand Whites, 74 male New Zealand Whites and six male Californian rabbits) weighing between 2.0 and 4.0 kg (mean weight  $\pm$  standard error =  $2.9 \pm 0.1$  kg). Thirty-two recordings of afferent chemoreceptor activity (three single and 29 multiple units) were obtained in 26 of these animals (all male; 24 New Zealand White and two Californian rabbits), i.e. recordings of chemoreceptor activity were obtained in approximately 30 per cent of the experimental animals. This is a low success rate compared to experiments on cats (Section

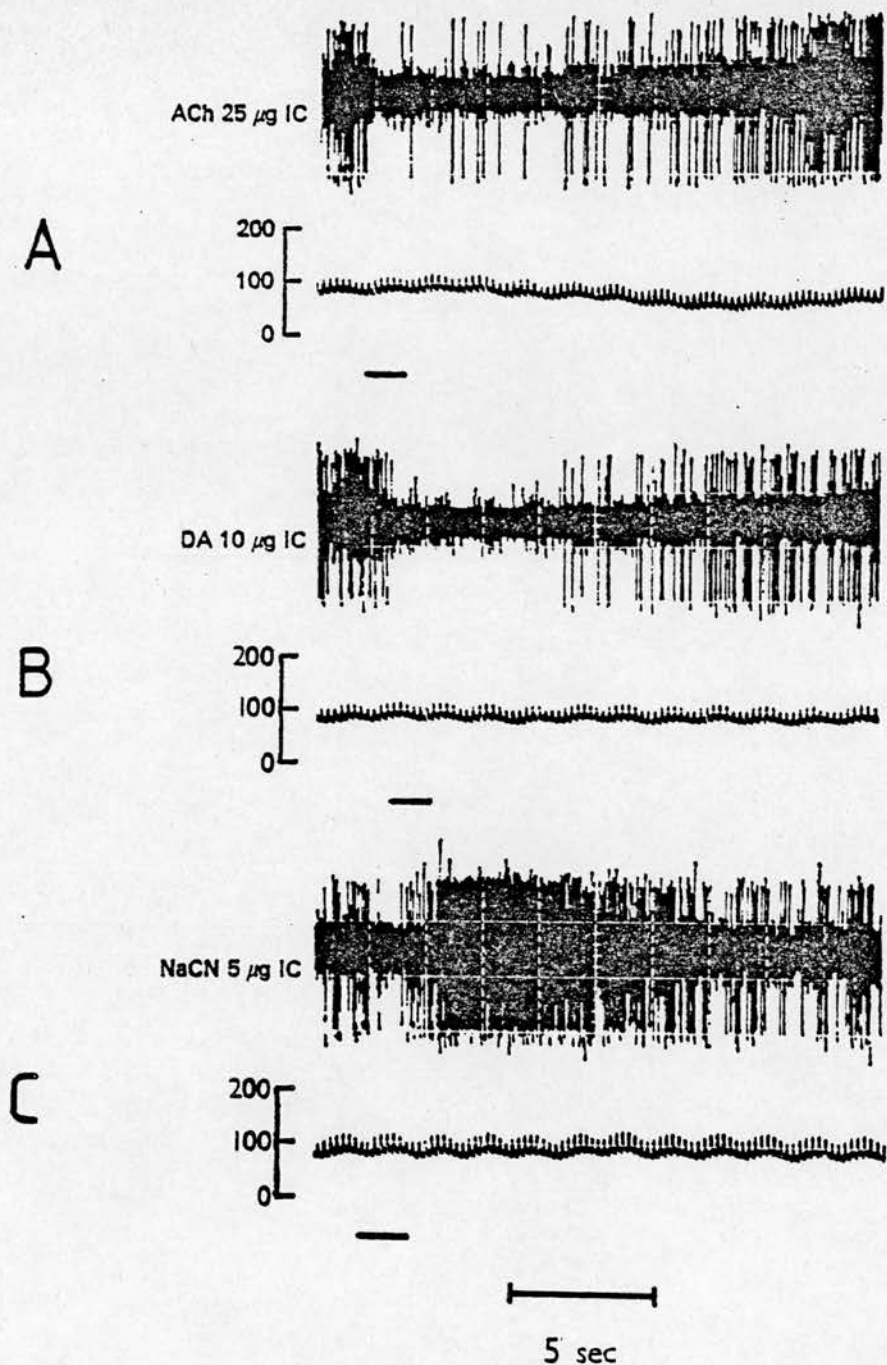


Figure 4.1

Recording of chemoreceptor activity obtained from a rabbit illustrating responses of the units to ACh (A), DA (B) and NaCN (C). Panels show, from above downwards : action potentials, B.P. (mm Hg) and injection marker. The time bar refers to all three panels.

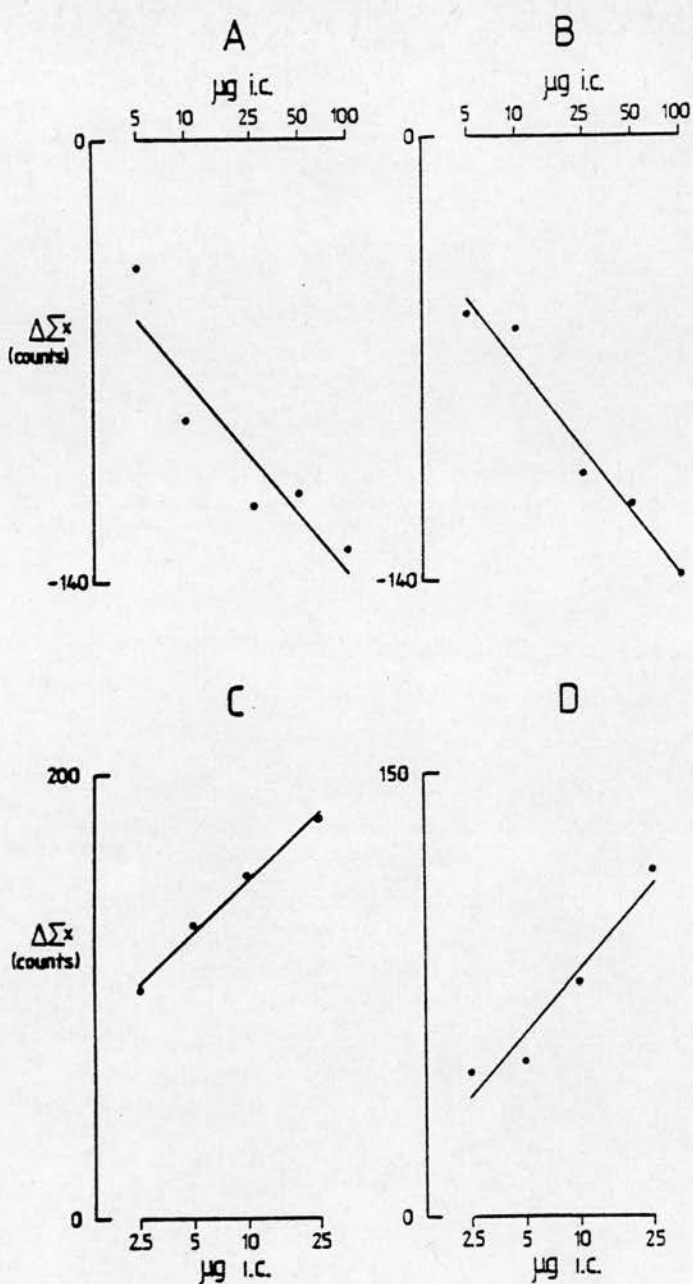


Figure 4.2

Dose-response data for ACh (A & B) and NaCN (C & D) obtained from four experiments. Panels A and C show data obtained from experiments in which the ganglioglomerular nerve was intact and panels B and D show data from experiments in which this nerve was eliminated. Data in A, B, C and D were from recordings of three, two, three and two chemoreceptor units respectively. Doses are plotted on a  $\log_{10}$  scale and lines fitted to the data by the method of least squares.

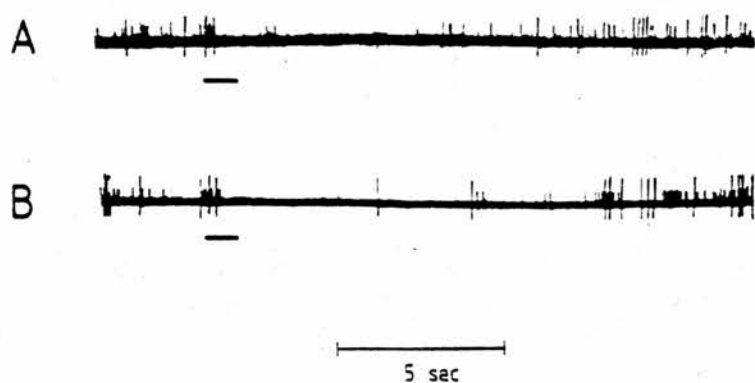


III) where recordings of chemoreceptor activity were obtained in approximately 90 per cent of the experimental animals. Possible reasons for the low success rate in rabbits and a discussion of non-chemoreceptor afferent activity in the rabbit sinus nerve are given in Appendix I.

Animals were anaesthetized with either sodium pentobarbitone or urethane and  $\alpha$ -chloralose as described in Section II.

#### Responses to acetylcholine

In all the experiments in which recordings of chemoreceptor activity were obtained, including two experiments in which the superior cervical ganglion was extirpated and two in which the ganglio-glomerular sympathetic nerve was cut, injections of ACh (1 - 250  $\mu$ g I.C.) caused an immediate, short-lasting (< 30 sec) inhibition of chemoreceptor activity. A typical response to an I.C. injection of ACh is illustrated in Figure 4.1A. Figure 4.2A, B, shows dose-response data for ACh in two experiments, one in which the ganglio-glomerular nerve was intact and one in which it was sectioned. There was an approximately linear relationship between  $\log_{10}$  dose and response ( $\Delta\Sigma x$ ) over the range 5 - 100  $\mu$ g I.C. (see Figure 4.2). Doses in this range, which always caused inhibition of chemoreceptor activity in the rabbit, are comparable to doses which stimulate chemoreceptor activity in the cat (McQueen, 1977). In some experiments, the inhibition observed following administration of high doses of ACh ( $\geq 50$   $\mu$ g I.C.) was preceded by a slight (2 - 3 times control discharge), transient period of stimulation lasting less than one second (see Figure 4.4C).



**Figure 4.3**

Panels A and B show chemoreceptor units from an experiment illustrating the response to ACh (10  $\mu\text{g}$  I.C.) before (A) and after (B) administration of gallamine (3 mg/kg I.V.) and panel C shows dose-response data from a second experiment (2 units) before (o---o) and after (x---x) administration of gallamine (6 mg/kg I.V.). Panels A and B show, from above downwards : action potentials and injection marker. The time bar refers to both A and B. In C doses were plotted on a log<sub>10</sub> scale and lines were fitted by the method of least squares.

### Response to sodium cyanide

NaCN increased carotid chemosensory activity in all the experiments in which recordings of chemoreceptor activity were obtained including the four experiments in which connections between the superior cervical ganglion and the carotid body were eliminated. The threshold dose for stimulation was about 2.5  $\mu\text{g}$  I.C. and a maximum response was elicited by 25  $\mu\text{g}$  I.C. The effective dose-range is comparable to that for exciting carotid chemoreceptors in the cat (McQueen, 1977). Figure 4.2C, D, shows dose response data for NaCN in two experiments, one in which the ganglio-glomerular nerve was intact and one in which this nerve had been cut, illustrating the approximately linear relationship between  $\log_{10}$  dose and response ( $\Delta\Sigma x$ ) over this range. A typical neurogram illustrating the response of rabbit carotid chemoreceptors to NaCN is shown in Figure 4.1C).

### Effects of Gallamine

It has been found that neuromuscular blocking agents such as gallamine and D-TC depress the stimulant effect of ACh on chemoreceptor activity in the cat carotid body *in vitro* (Eyzaguirre and Zapata, 1965b). In all the experiments performed in the present study, gallamine triethiodide (3 mg/kg I.V.) was administered to paralyse spontaneous respiration and minimise muscular movement in the vicinity of the recording electrodes (see Section II). Two experiments were performed in order to ascertain whether low doses of gallamine had any effect on the response of the rabbit carotid chemoreceptors to ACh or NaCN. Gallamine (3-6 mg/kg I.V.) had no effect on the chemoreceptor response to NaCN (see Figure 4.3C).

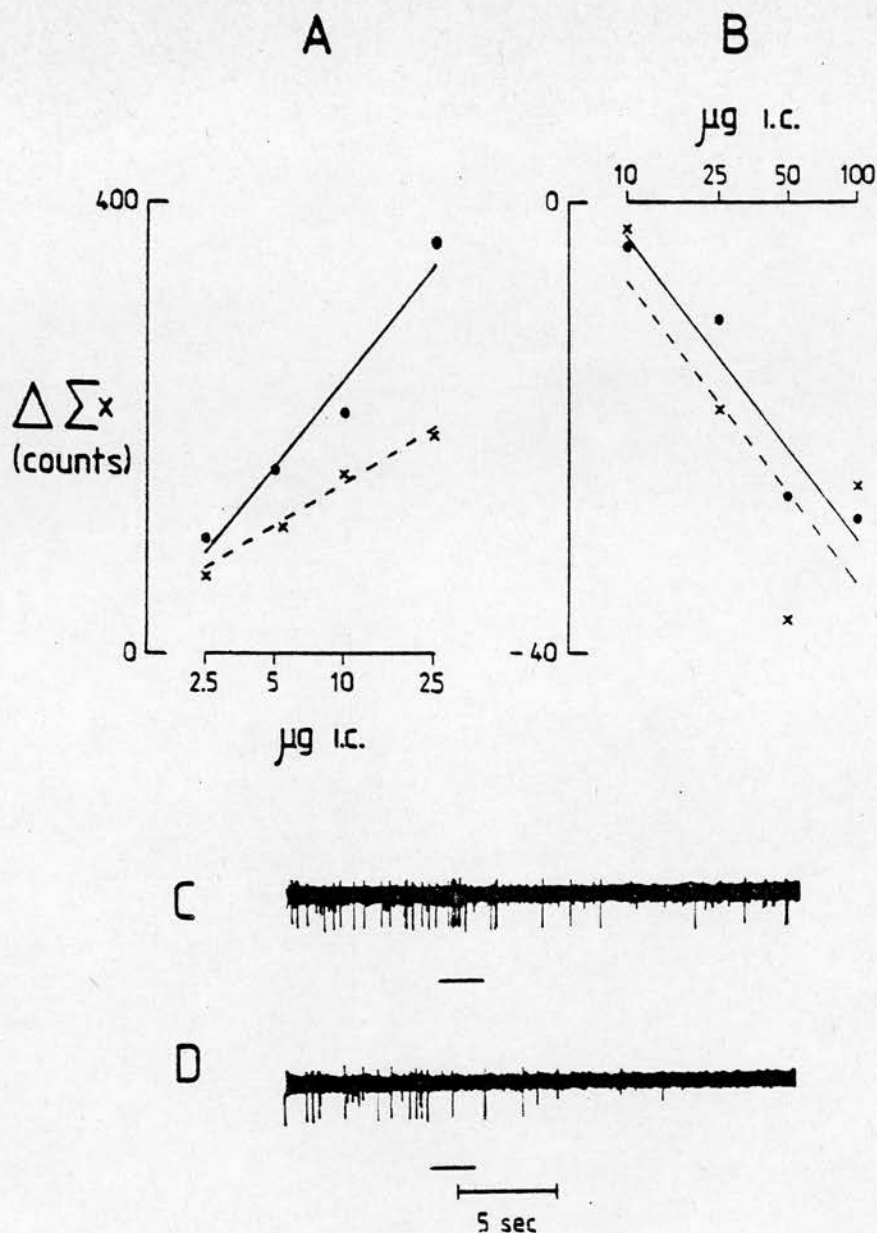


Figure 4.4

Panels A and B show dose-response data for NaCN (A) and ACh (B), before (●—●) and after (x---x) administration of mecamlamine (0.5 mg /kg I.C.). Doses are plotted on a  $\log_{10}$  scale and lines fitted by the method of least squares. Panels C and D show the chemoreceptor response to a high dose (50  $\mu g$  I.C.) of ACh, before (C) and after (D) administration of mecamlamine (0.5 mg /kg I.C.). The chemoreceptor recording shown in C and D is from the same experiment as the data shown in A and B. Panels C and D show from above downwards : action potentials and injection marker. The time bar refers to panels C and D.

It was not possible to obtain linearly related dose-response data for ACh before and after administration of gallamine since injection of high doses of ACh ( $\geq 25 \mu\text{g}$  I.C.) prior to administration of gallamine usually resulted in loss of the recording due to muscular movement causing displacement of the nerve from the recording electrodes. The inhibition of chemoreceptor activity produced by small doses of ACh (5 or 10  $\mu\text{g}$  I.C.) was not, however, appreciably affected by gallamine (see Figure 4.3A, B).

#### Effects of mecamlamine

Mecamlamine is a potent ganglion blocking drug (Stone, Torchiana, Navarro and Beyer, 1956; Bennet, Tyler and Zaimis, 1957) which can reduce or abolish the increase in chemoreceptor activity evoked by ACh in the cat carotid body *in vitro* (Eyzaguirre and Zapata, 1968b) or *in vivo* (Sampson, 1971; Nishi and Eyzaguirre, 1971).

In the present experiments with mecamlamine, the appropriate volume of dextran solution (2.5% dextran, 5% glucose in distilled water) required to maintain B.P. at the control level was administered I.V. This treatment prevented the fall in B.P. which would otherwise have accompanied administration of this ganglion blocking, drug making interpretation of results difficult. The effects of mecamlamine on carotid chemoreceptor activity were investigated in four experiments.

Mecamlamine (0.5 - 5 mg/kg I.V. or 0.25 - 0.50 mg/kg I.C.) did not reduce the inhibitory chemoreceptor response to ACh but caused a slight potentiation of responses in some experiments (see Figure 4.4B). The transient excitatory effect of ACh, when observed, was



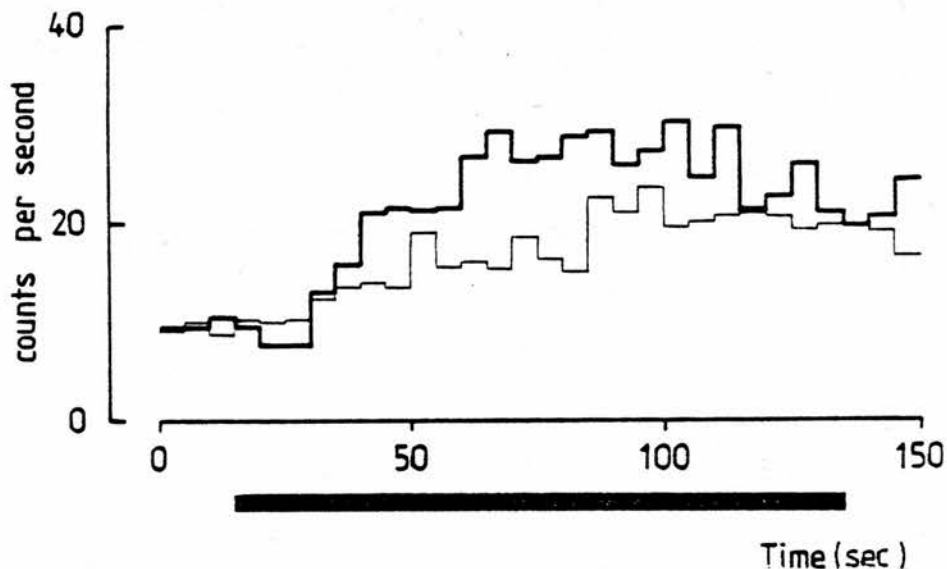


Figure 4.5

Response of carotid chemoreceptors (two units) to 120 sec of hypoxia (breathing 5% O<sub>2</sub> in N<sub>2</sub>) before (—) and after (---) administration of mecamlamine (1.0 mg /kg I.V.). Panels show, from above downwards : discharge frequency (averaged over 5 sec intervals), time and period of hypoxia (horizontal black bar)..

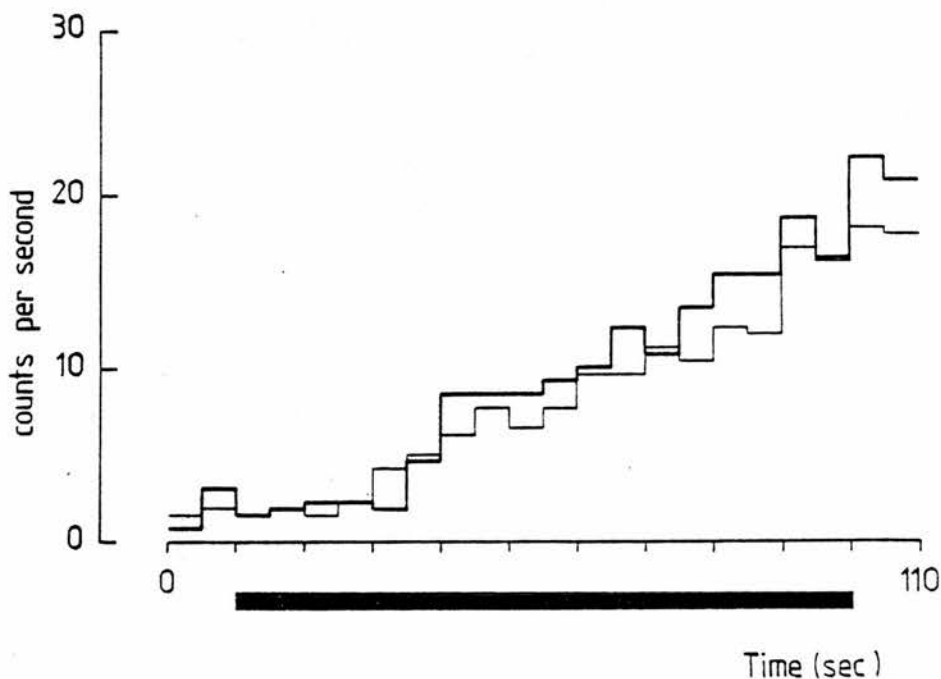


Figure 4.6

Response of carotid chemoreceptors (three units) to 90 sec of hypoxia (breathing 5% O<sub>2</sub> in N<sub>2</sub>) before (—) and after (---) administration of atropine (0.5 mg /kg I.C.). Panels show, from above downwards : discharge frequency (averaged over 5 sec intervals), time and period of hypoxia (horizontal black bar)..

abolished by mecamlamine (see Figure 4.4C,D). The response of the carotid chemoreceptors to NaCN, especially the higher doses, was reduced but not abolished by mecamlamine (see Figure 4.4A).

Mecamlamine also caused a slight reduction in the chemoreceptor response to hypoxia (see Figure 4.5). There was no obvious change in the frequency of spontaneous discharge in animals breathing room air following I.V. injection of mecamlamine (1 - 5 mg/kg) but there was a reduced frequency of discharge following close-arterial administration of the drug (0.5 mg/kg I.C.) (see Table 4.1).

TABLE 4.1: Spontaneous chemoreceptor discharge frequency ( $\bar{x}$ , counts/sec) in animals breathing room air, before and after administration of mecamlamine. In experiments marked \*, progressively larger doses of mecamlamine were administered. In these cases the dose included in the table is the highest dose administered

Experiment code	No. of units	Dose of mecamlamine	$\bar{x}$ before	$\bar{x}$ after
R63	3	1 mg/kg I.V.	6.7	7.6
R42*	3	2 mg/kg I.V.	21.9	19.0
R37	6	5 mg/kg I.V.	19.8	24.3
R61*	1	0.5 mg/kg I.C.	3.0	0.6

#### Effect of atropine

The effect of atropine on chemoreceptor activity was studied in nine experiments. There was considerable variation in the dose of atropine required to block the vasodepressor action of ACh. Low doses of atropine (cumulative doses of 1 and 2 mg/kg I.V.) had no consistent effect on either the depressor response or the inhibition of chemoreceptor activity produced by ACh. The mean dose-ratio (see Table 4.2) for the 1 and 2 mg/kg I.V. doses indicate an overall

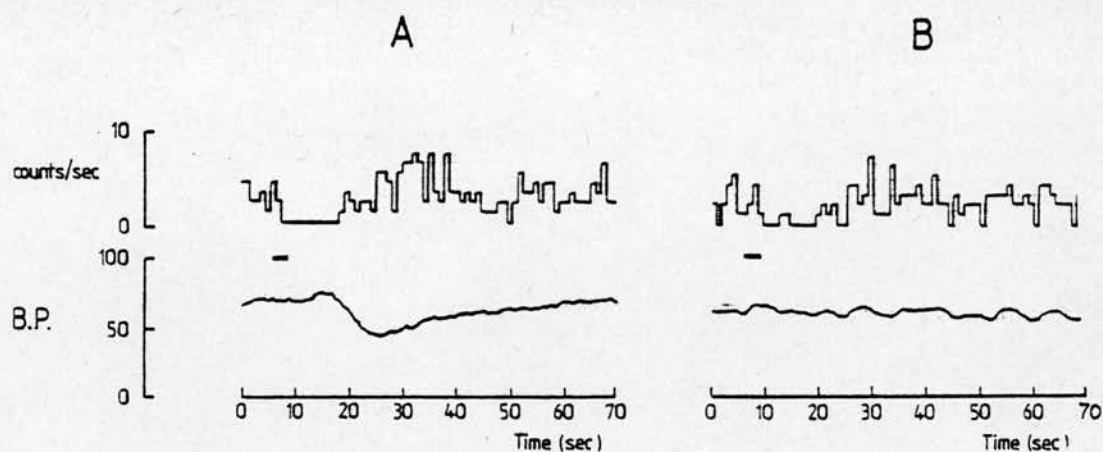


Figure 4.7

Response of carotid chemoreceptors (three units) to ACh (10  $\mu$ g I.C.) before (A) and after (B) administration of atropine (10 mg /kg I.V.). Panels show from above downwards : discharge frequency, injection marker, mean B.P. (mm Hg) and time.

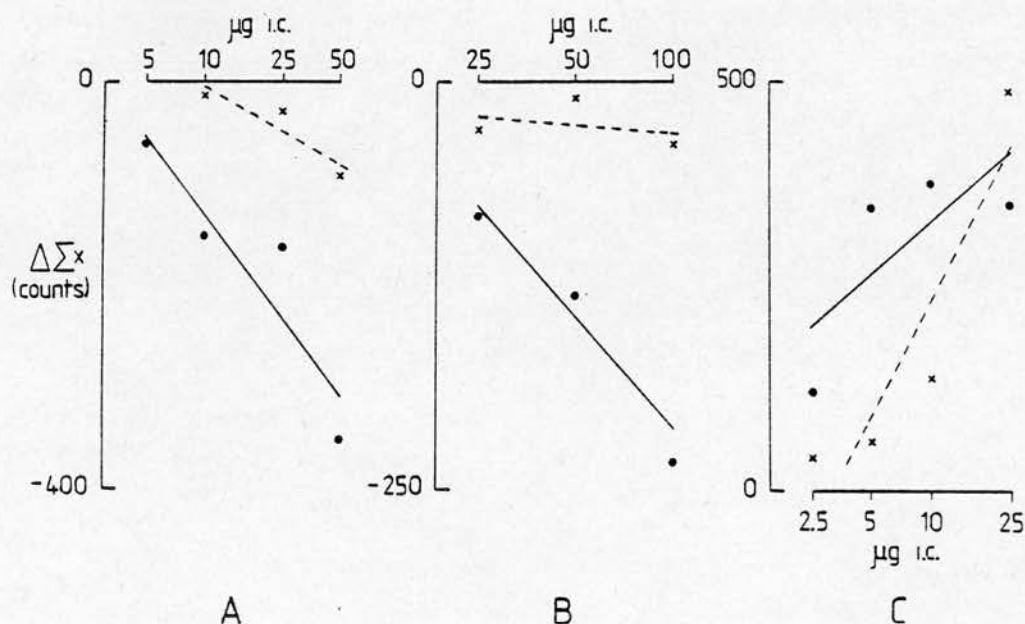


Figure 4.8

Dose-response data for ACh (A), BCh (B) and NaCN (C), from a recording of three chemoreceptor units, before (●—●) and after (x---x) administration of atropine (1.0 mg /kg I.C.). Doses are plotted on a log<sub>10</sub> scale and lines fitted by the method of least squares.

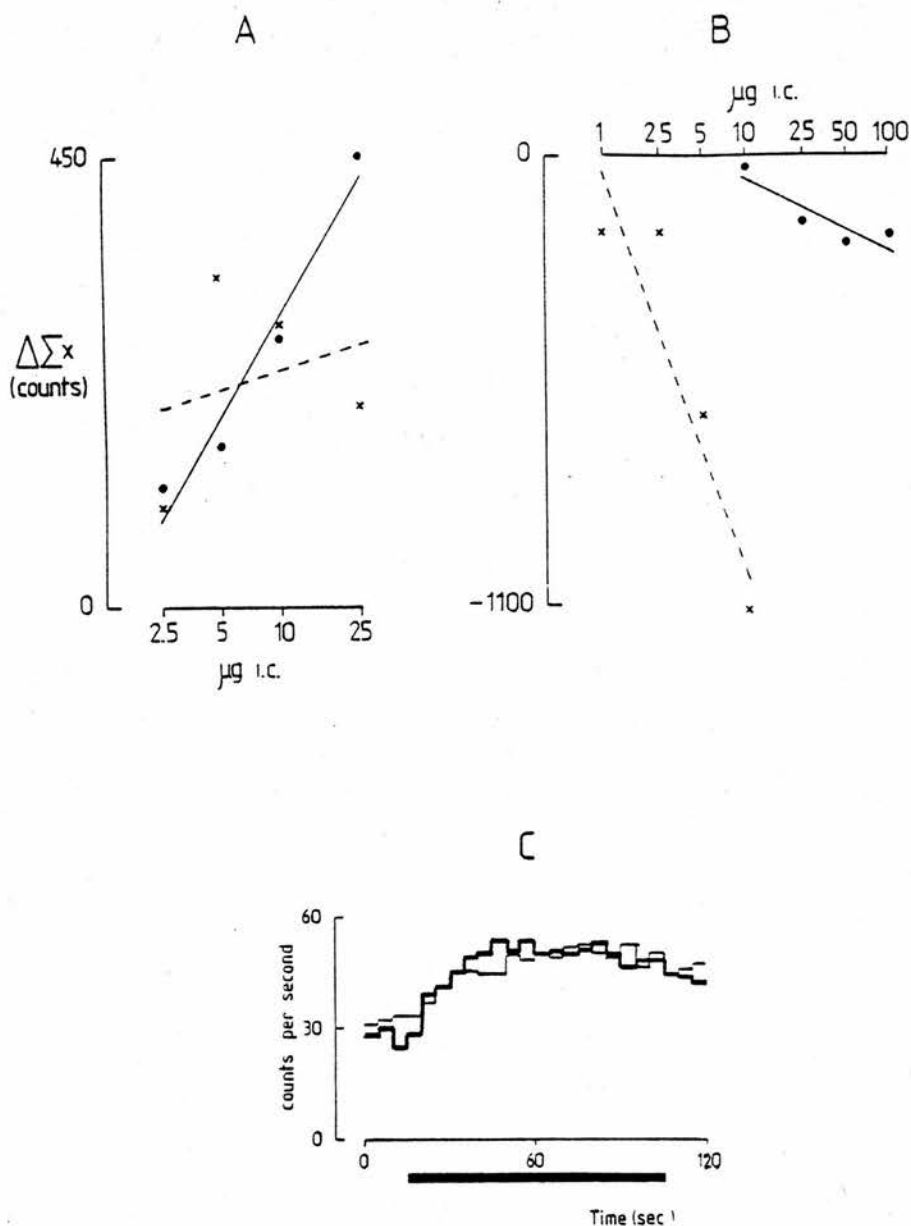
tendency for these low doses of atropine to reduce the inhibitory effect of ACh although the high value of the range associated with each mean dose-ratio illustrates the variability of this effect.

TABLE 4.2: Geometric mean dose-ratios (d.r.) for the effects of atropine on chemoreceptor responses to ACh. The number of experiments (n), the median (m) and range of values (r) associated with each ratio are included in the table

Dose of atropine	d.r.	n	m	r
1 mg/kg I.V.	1.7	4	1.9	4.0
2 mg/kg I.V.	2.2	3	1.9	5.8
5 mg/kg I.V.	5.7	3	9.2	19.4

Higher doses of atropine (5 mg/kg I.V. additional to the 1 and 2 mg/kg I.V. doses or 10 mg/kg I.V.) sufficient to block the depressor effects of ACh reduced, but did not abolish, the inhibition of chemoreceptor activity produced by ACh (see Figure 4.7) although, as with the lower doses of atropine, the magnitude of the effect varied considerably between experiments. Administration of atropine close-arterial to the carotid body (1 mg/kg I.C.), however, greatly reduced the chemoreceptor response to ACh (see Figure 4.8A).

Chemoreceptor responses to NaCN were not appreciably altered following I.V. administration of atropine (1 - 10 mg/kg I.V.). Sufficient data was available to calculate the geometric mean dose-ratio for the 1 and 2 mg/kg I.V. doses of atropine on the response to NaCN giving values of 1.4 (number of experiments = 4, median = 1.2, range = 2.6) and 1.0 (number of experiments = 3, median = 0.9, range = 1.1) respectively. There was a slight reduction in the response to NaCN following close-arterial administration of atropine



**Figure 4.9**

Panels A and B show dose-response data for NaCN (A) and ACh (B), from a recording of four chemoreceptor units, before (●—●) and after (x---x) administration of physostigmine (0.1 mg/kg i.c.). Doses are plotted on a log<sub>10</sub> scale and lines fitted by the method of least squares. Panel C shows the response to hypoxia (breathing 5% O<sub>2</sub> in N<sub>2</sub>), in the same experiment, before (—) and after (---) administration of physostigmine (0.1 mg/kg i.c.). Panel C shows from above downwards: discharge frequency, time and period of hypoxia (horizontal black bar).



(1 mg/kg I.C.) (see Figure 4.8C). The chemoreceptor response to hypoxia did not appear to be affected by atropine (see Figure 4.6) nor was there any obvious change in the frequency of chemoreceptor discharge in animals breathing room air (see Table 4.3).

TABLE 4.3: Spontaneous chemoreceptor discharge frequency ( $\bar{x}$ , counts/sec - values quoted as arithmetic mean  $\pm$  standard error) in animals breathing room air before and after administration of atropine. Doses marked \* were from the same experiment, 0.5 mg/kg I.C. being administered subsequent to 10 mg/kg I.V.

No. of experiments	Dose of atropine	$\bar{x}$ before	$\bar{x}$ after
5	1 mg/kg I.V.	$19.2 \pm 4.3$	$15.1 \pm 3.3$
4	2 mg/kg I.V.	$16.5 \pm 5.3$	$9.4 \pm 4.5$
3	5 mg/kg I.V.	$16.7 \pm 4.0$	$18.1 \pm 2.5$
1	10 mg/kg I.V.*	0.6	0.4
1	0.5 mg/kg I.V.*	0.6	0.9
2	1.0 mg/kg I.V.	$21.4 \pm 2.4$	$17.0 \pm 1.6$

#### Effects of physostigmine (eserine)

Physostigmine is a potent anticholinesterase drug which is effective against both acetylcholinesterase and pseudocholinesterase (see Koelle and Gilman, 1949). The effects of physostigmine on carotid chemoreceptor activity were studied in three rabbits. In each of these animals the ganglio-glomerular nerve remained intact.

Administration of cumulative doses of 0.1 and 0.2 mg/kg I.C. of physostigmine greatly potentiated ACh-induced inhibition of chemoreceptor activity (see Figure 4.9B). There was no increase in the tendency for ACh to produce excitatory effects following administration of physostigmine. The excitatory response to NaCN

became less consistent following administration of physostigmine making the effects of the drug difficult to interpret (see Figure 4.9A). There was, however, no consistent change in the magnitude of responses to NaCN following administration of physostigmine. The chemoreceptor response to hypoxia was not appreciably altered by physostigmine (see Figure 4.9C). Physostigmine sometimes increased and sometimes decreased the frequency of spontaneous discharge in animals breathing room air but had no consistent effect (see Table 4.4).

TABLE 4.4: Spontaneous chemoreceptor discharge frequency ( $\bar{x}$ ) in animals breathing room air, before and after administration of cumulative doses of 0.1 and 0.25 mg/kg I.C. of physostigmine

Experiment code	No. of units	x (counts/sec):		
		control	after 0.1 mg/kg I.C.	after 0.2 mg/kg I.C.
R76	3	25.6	20.0	-
R77	4	21.1	36.7	12.6
R85	6	81.7	92.7	77.9

#### Response to suberyldicholine (SDCh)

SDCh is a potent nicotinic agonist which has been shown to stimulate the carotid chemoreceptors of the cat (Anichkov and Belen'kii, 1963; Dardymov and Ger, 1964; McQueen, 1974). The effects of SDCh on carotid chemoreceptor activity in the rabbit was studied in four experiments. SDCh (10 - 100  $\mu$ g I.C.) had no effect or caused a slight inhibition of chemoreceptor activity in this species, but had no consistent effect (see Figure 4.10).

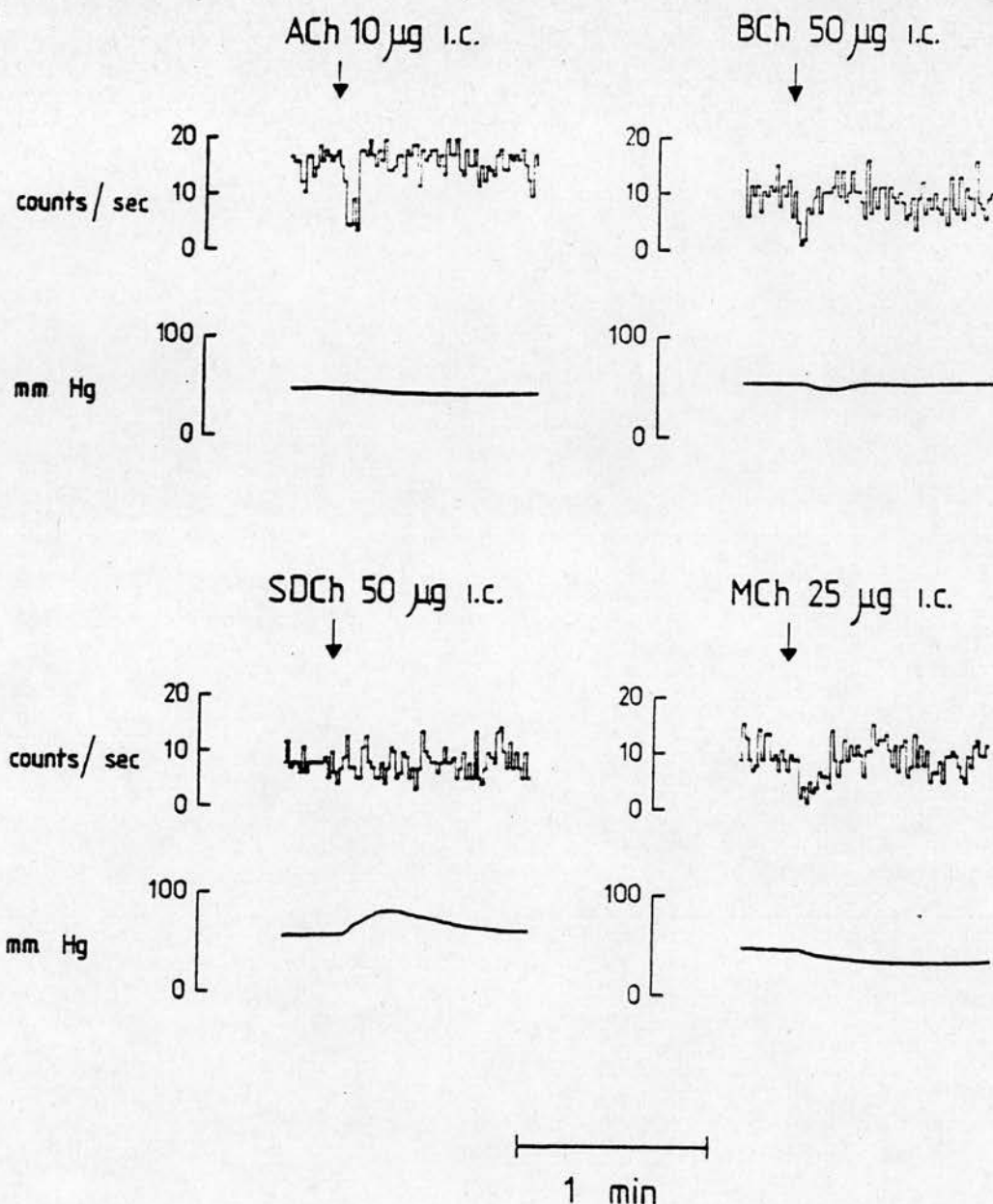


Figure 4.10

Responses of carotid chemoreceptors, from a recording of three units, to ACh, BCh, SDCh and MCh. Panels show, from above downwards : discharge frequency and mean B.P. Injections were made at the arrows. The time bar refers to all four panels.

#### Response to bethanechol (BCh)

BCh is a muscarinic cholinomimetic substance which is free of nicotinic actions (Molitor, 1936). Injections of single doses of BCh (50 or 100  $\mu\text{g}$  I.C.) caused inhibition of chemoreceptor activity in each of the five rabbits in which its action was investigated (see Figure 4.10). The relationship between  $\log_{10}$  dose and response was studied in one experiment and was found to be linear in the range 25 - 100  $\mu\text{g}$  I.C. (see Figure 4.8B). Unlike the response to ACh, the inhibition of chemoreceptor activity produced by BCh was not preceded by stimulation at high doses (i.e. 50 - 100  $\mu\text{g}$  I.C.). The chemoreceptor response to BCh was greatly reduced following close-arterial administration of atropine (1 mg/kg I.C.) (see Figure 4.8B).

#### Response to methacholine (MCh)

MCh is a potent cholinergic agonist which exhibits predominantly muscarinic cholinomimetic effects (Simonaert, 1932; Hunt and Renshaw, 1934) although it can stimulate carotid chemoreceptors in the cat by a weak nicotinic cholinomimetic action (McQueen, 1978; see Section 1).

The effect of MCh on carotid chemoreceptor activity in the rabbit was studied in three experiments. Injections of single doses of MCh (5, 10 or 25  $\mu\text{g}$  I.C.) caused an inhibition of chemoreceptor activity which was qualitatively similar to that produced by ACh or BCh (see Figure 4.10).

There was considerable variation between experiments in the relative potencies of ACh, BCh and MCh in their ability to produce inhibition of chemoreceptor activity and insufficient data are available to make a quantitative estimate of their relative potency.

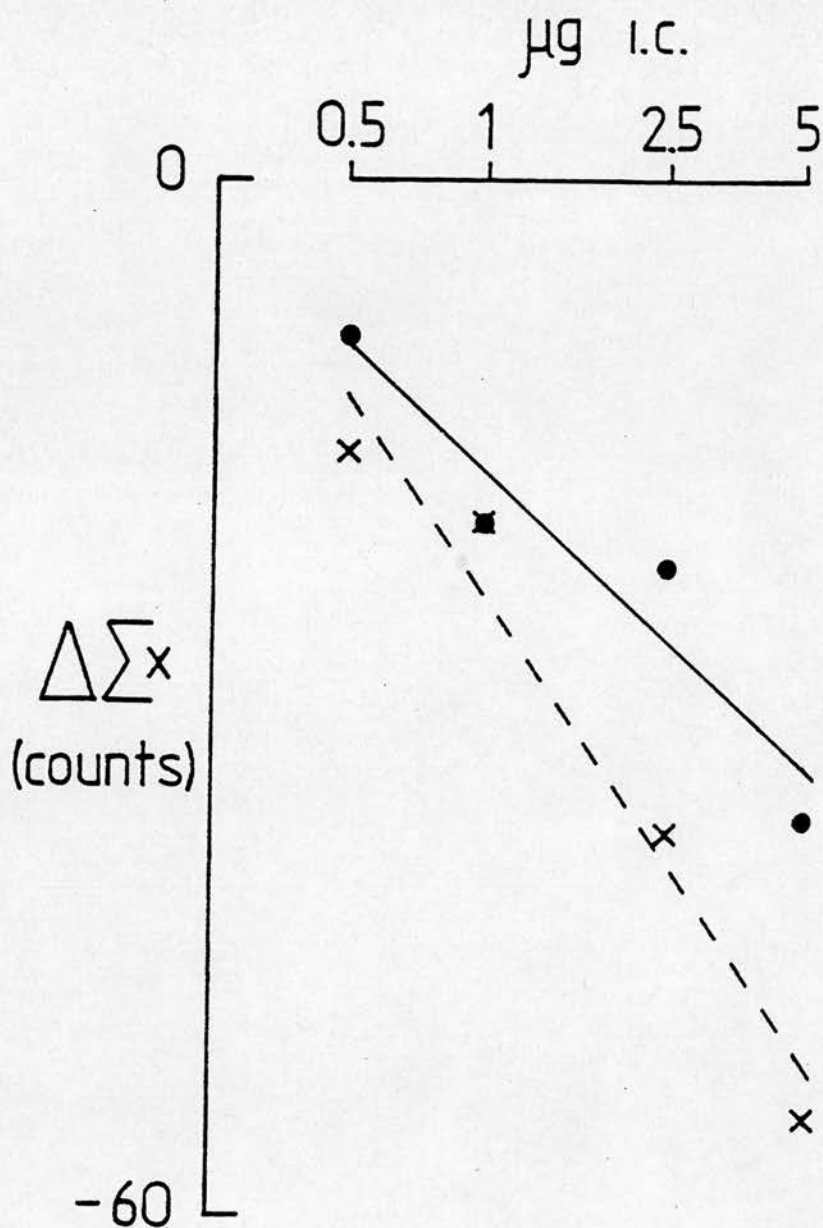
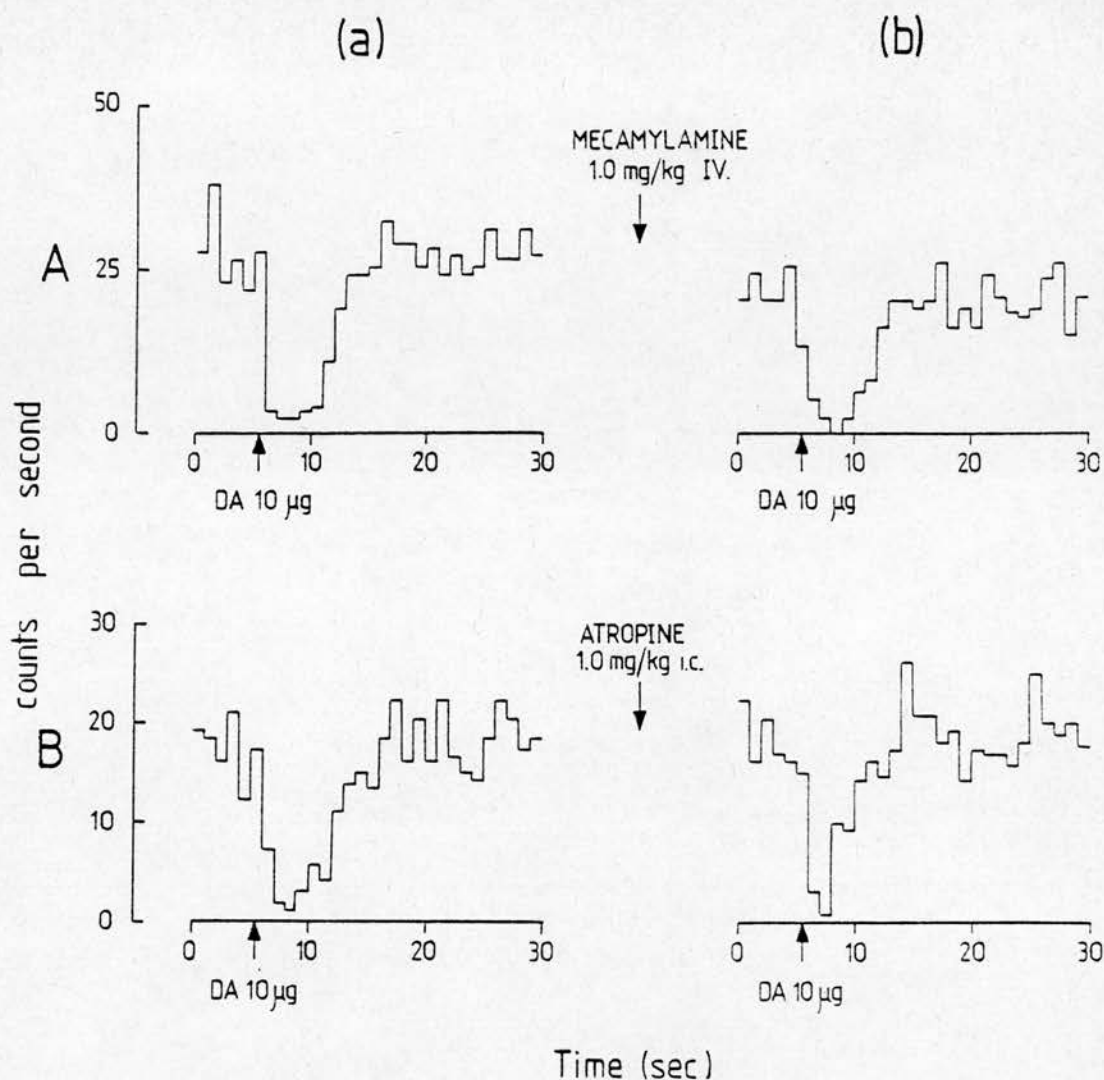


Figure 4.11

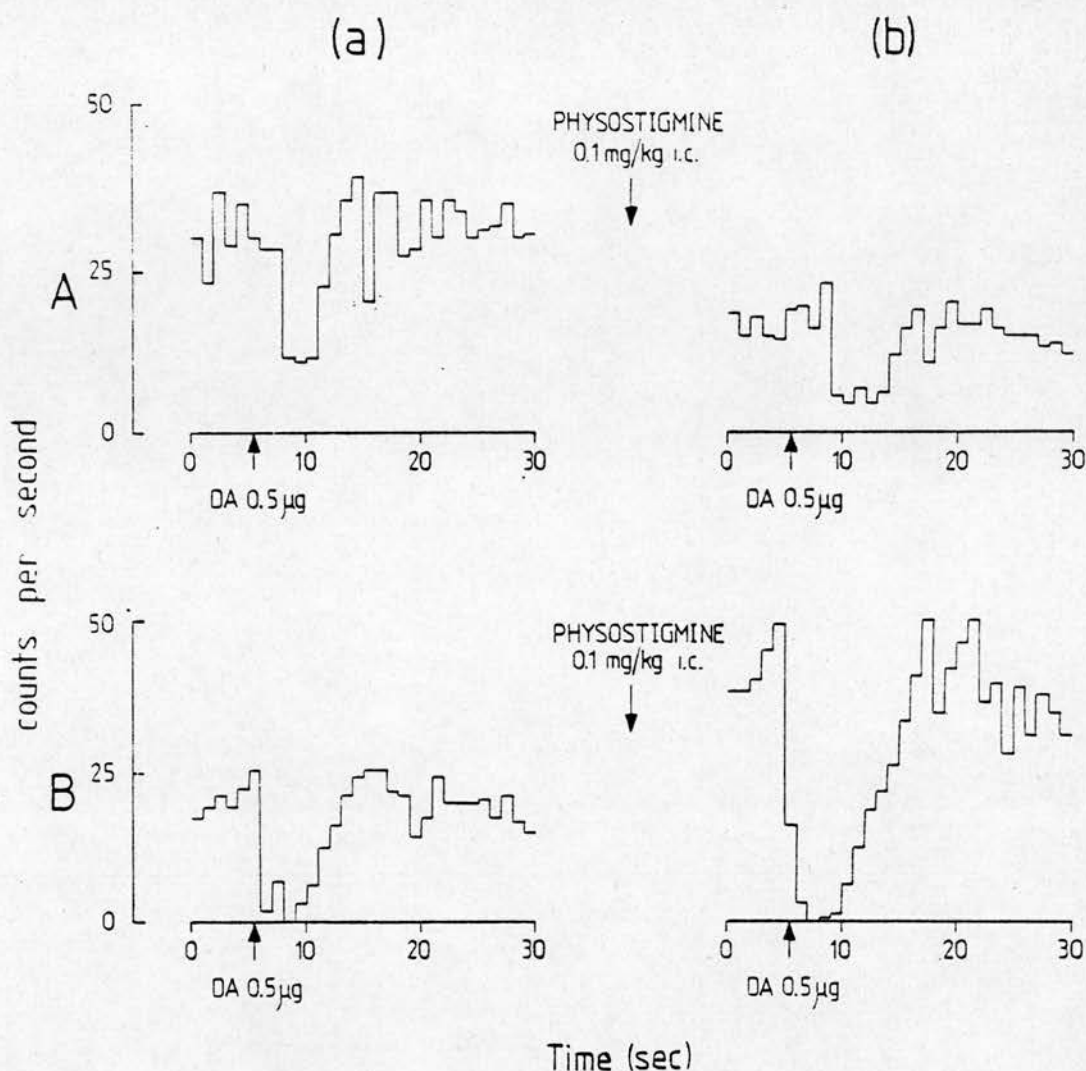
Dose-response data for DA, from a recording of one chemoreceptor unit, before (●—●) and after (x---x) administration of gallamine (3.0 mg /kg I.V.). Doses are plotted on a  $\log_{10}$  scale and lines fitted by the method of least squares.





**Figure 4.12**

Data from two experiments showing the response of carotid chemoreceptors to DA before (a) and after (b) administration of mecamylamine (A) and atropine (B). Recordings were of three chemoreceptor units in both experiments. Panels show, from above downwards : discharge frequency and time. Injections of DA were made at the upward pointing arrows.



**Figure 4.13**

Data from two experiments showing the response of carotid chemoreceptors to DA before (a) and after (b) administration of physostigmine. Recordings were of three units in A and four units in B. Panels show from above downwards : discharge frequency and time. Injections of DA were made at the upward pointing arrows.

Qualitatively, however, on a molar basis, the order of potency was MCh  $\geq$  ACh > BCh.

#### Responses to dopamine

The effect of DA on carotid chemoreceptor activity was studied in 22 experiments. Injections of DA (0.5 - 10  $\mu$ g I.C.) caused an immediate, short-lasting (< 30 sec) depression of chemoreceptor activity in all the experiments. Figure 4.1B shows a typical response of rabbit carotid chemoreceptors to DA. The present study was restricted to low doses of DA since it had been found in experiments with cats that higher doses of DA tend to produce secondary excitations which curtail the primary inhibition and give inconsistent responses (see Section III). In some experiments, however, the inhibitory response of the rabbit chemoreceptors to DA was followed by a slight secondary excitation even at these low doses. This phenomenon was uncommon, occurring in response to only 12 of a total of 50 injections of DA.

The relationship between the inhibitory response to DA and  $\log_{10}$  dose was studied in eight experiments and was found to be approximately linear over the range 0.5 - 5  $\mu$ g I.C. Gallamine (3 - 6 mg/kg I.V.) slightly potentiated the inhibitory response to DA (see Figure 4.11). There was no apparent change in the response to single doses of DA (5 or 10  $\mu$ g I.C.) following administration of mecamylamine (0.5 - 5 mg/kg I.V. or 0.25 - 0.5 mg/kg I.C.) (see Figure 4.12A) or low doses of atropine (1 - 10 mg/kg I.V.). There was, however, a slight reduction in the response to DA following close-arterial administration of atropine (1.0 mg/kg I.C.) (see Figure 4.12B). The effects of physostigmine on the response to DA

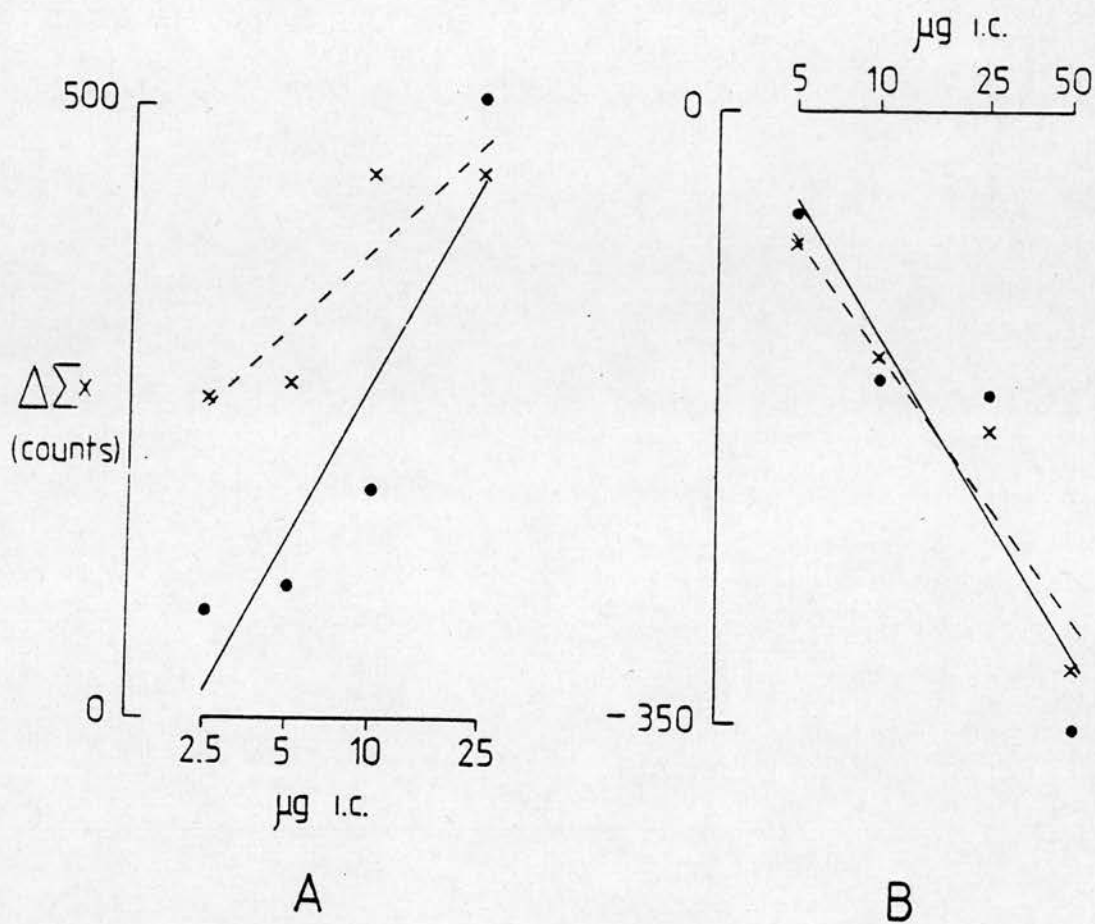


Figure 4.14

Dose-response data for NaCN (A) and ACh (B), obtained from a recording of three chemoreceptor units, before (●—●) and after (x—x) administration of  $\alpha$ -flupenthixol (0.25 mg /kg I.V.). Doses are plotted on a  $\log_{10}$  scale and lines fitted by the method of least squares.

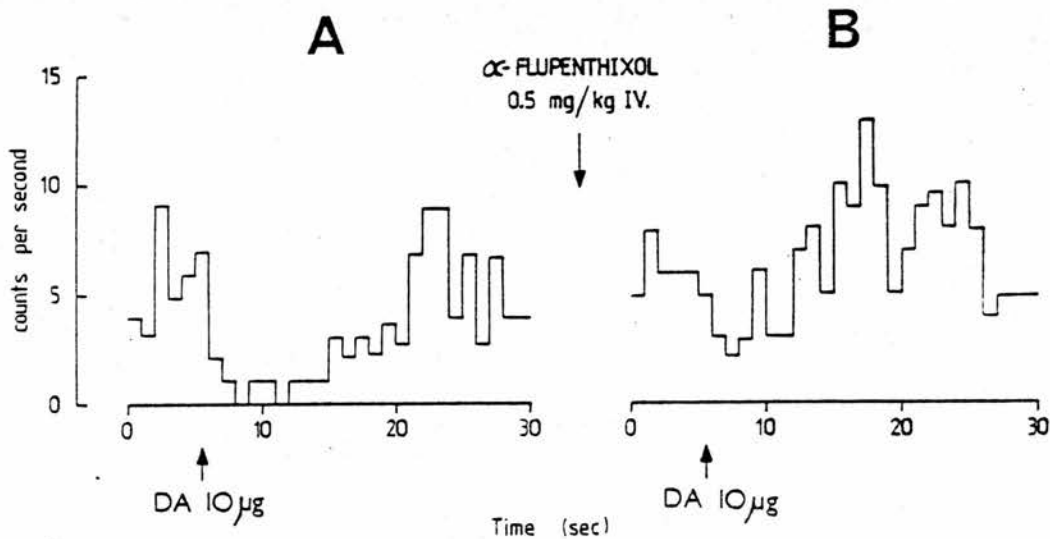


Figure 4.15

Response of carotid chemoreceptors (four units) to DA before (A) and after (B) administration of  $\alpha$ -flupenthixol. Panels show, from above downwards : discharge frequency and time. DA was injected at the upward pointing arrows.

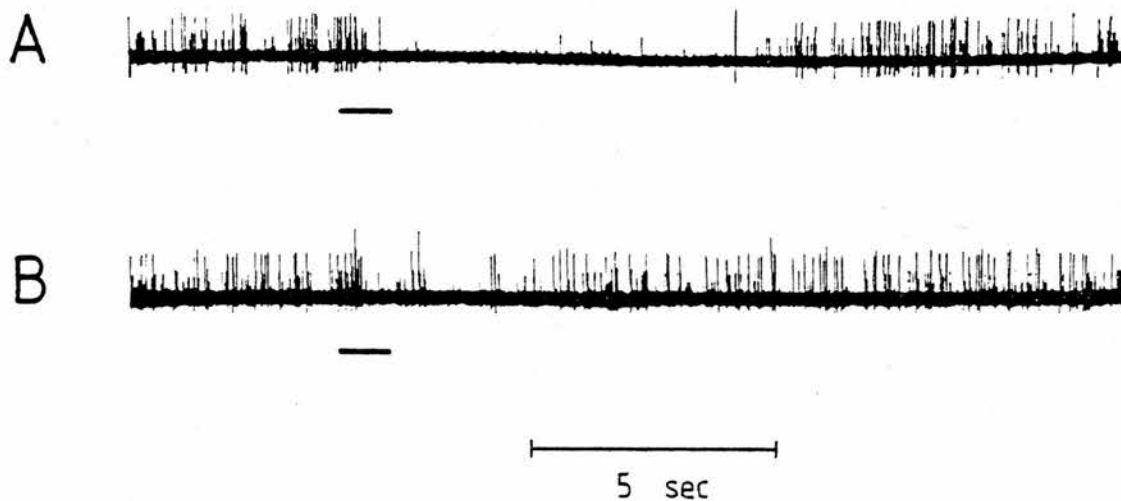


Figure 4.16

Chemoreceptor units from an experiment showing the response to D-amphetamine (10  $\mu$ g I.C.) before (A) and after (B) administration of  $\alpha$ -flupenthixol (0.5 mg /kg I.V.). Panels show, from above downwards : action potentials and injection marker. The time bar refers to both panels.



were studied in two experiments. The results obtained are difficult to interpret, however, because of the changes in spontaneous discharge frequency which occurred following administration of physostigmine. In one experiment there was no change in the numerical value ( $\Delta\Sigma x$ ) of the response to DA (see Figure 4.13A) while in the second this was increased (see Figure 4.13B). In both experiments the duration of the response to DA was increased which suggests that physostigmine may slightly potentiate the effects of DA.

#### Effects of $\alpha$ -flupenthixol

The effect of  $\alpha$ -flupenthixol (0.25 - 0.5 mg/kg I.V. or 0.25 - 0.5 mg/kg I.C.) on chemoreceptor activity was studied in seven rabbits. The inhibitory action of DA on carotid chemosensory

TABLE 4.4: Spontaneous chemoreceptor discharge frequency ( $\bar{x}$ , counts/sec - presented as arithmetic mean  $\pm$  standard error) in animals breathing room air before and after administration of  $\alpha$ -flupenthixol

No. of experiments	Dose of $\alpha$ -flupenthixol	$\bar{x}$ before	$\bar{x}$ after
2	0.25 mg/kg I.V.	$26.5 \pm 2.7$	$20.9 \pm 0.8$
4	0.50 mg/kg I.V.	$15.3 \pm 6.6$	$16.2 \pm 6.9$
1	0.25 mg/kg I.C.	19.9	27.6
2	0.50 mg/kg I.C.	$26.3 \pm 6.5$	$30.7 \pm 6.4$

activity was reduced or abolished following administration of  $\alpha$ -flupenthixol and there was an increased tendency for DA to produce delayed excitatory effects (see Figure 4.15). The inhibitory action of ACh on chemosensory activity was little affected by  $\alpha$ -flupenthixol while the excitatory response to NaCN was potentiated (see Figure 4.14). There was no obvious change in

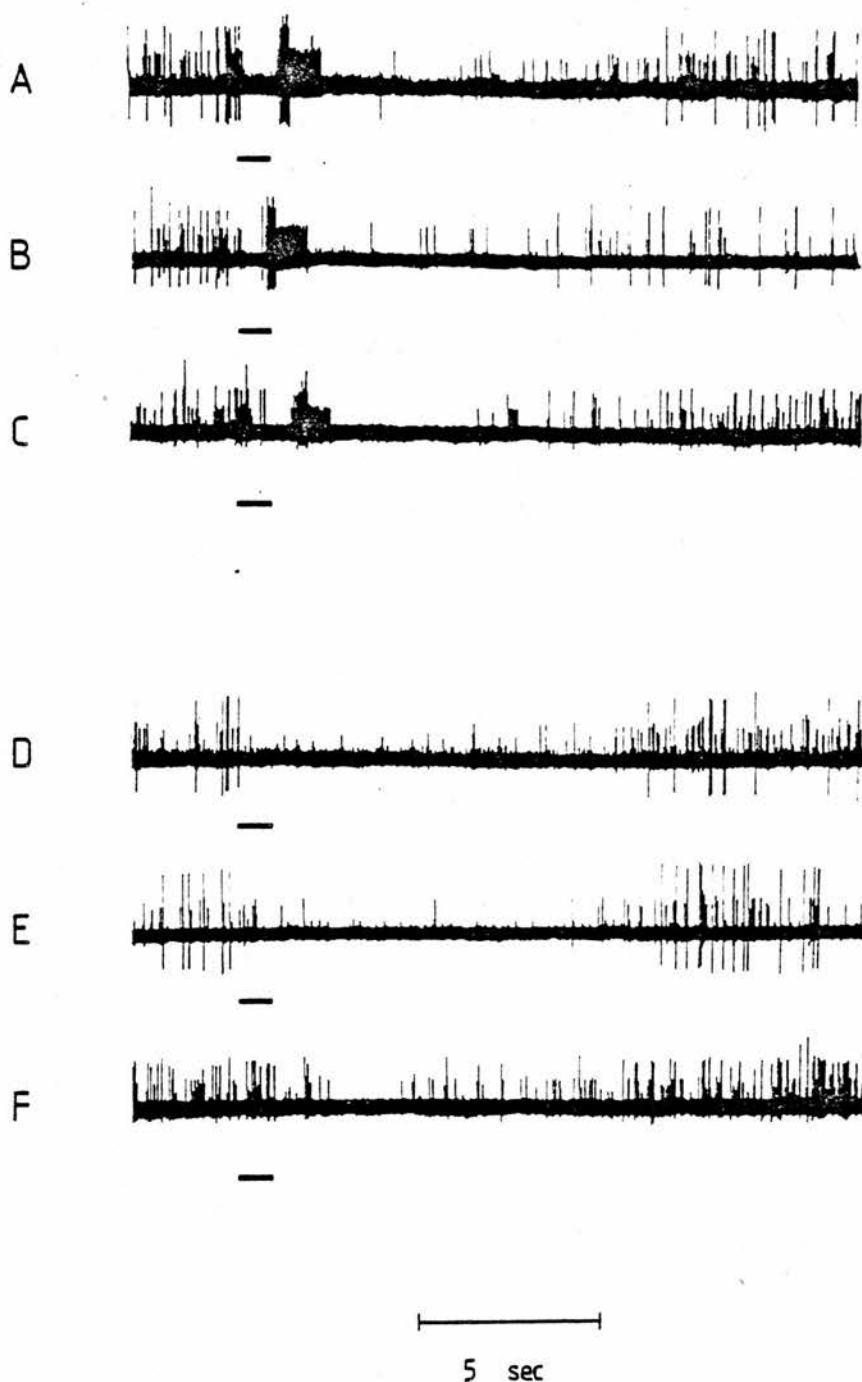


Figure 4.17

Chemoreceptor units from an experiment showing responses to 5-HT (5  $\mu$ g I.C.) (A, B & C) and DA (0.5  $\mu$ g I.C.) (D, E & F) before (A & D) and after (B & E) administration of gallamine (3.0 mg /kg I.V.) and after (C & F) subsequent administration of  $\alpha$ -flupenthixol (0.5 mg /kg I.V.). Panels show, from above downwards : action potentials and injection marker. The time bar refers to all six panels.

the frequency of spontaneous chemoreceptor discharge following administration of  $\alpha$ -flupenthixol (see Table 4.4).

#### Response to D-amphetamine

The effect of D-amphetamine on chemoreceptor activity in the rabbit was studied in one experiment. Injection of a single dose of D-amphetamine (10  $\mu$ g I.C.) caused an immediate depression of chemoreceptor activity which was greatly reduced by administration of  $\alpha$ -flupenthixol (0.5 mg/kg I.C.) (see Figure 4.16).

#### Responses to 5-hydroxytryptamine

The effect of 5-HT on carotid chemoreceptor activity was studied in 11 experiments. Injection of 5-HT (0.25 - 10  $\mu$ g I.C.) caused an intense but transient (< 2 sec) stimulation of chemoreceptor activity followed by a period of inhibition (2 - 25 sec) (see Figure 4.17). Both the excitatory and inhibitory components of the response were subject to considerable variation in any experiment making an accurate assessment of the effects of blocking drugs difficult. Gallamine (3 - 6 mg/kg I.V.) did not appear to modify the response to 5-HT (see Figure 4.17), nor did the cholinergic antagonists mecamlamine or atropine. Doses of  $\alpha$ -flupenthixol which greatly reduced the inhibitory effects of DA had little effect on responses to 5-HT (see Figure 4.17).

#### Responses to vasodilator substances

##### 1. Sodium nitrite

Sodium nitrite is a potent vasodilator which acts directly on vascular smooth muscle (Weiss, Wilkins and Haynes, 1937). Injection of sodium nitrite (25 - 100  $\mu$ g I.C.) had either no effect

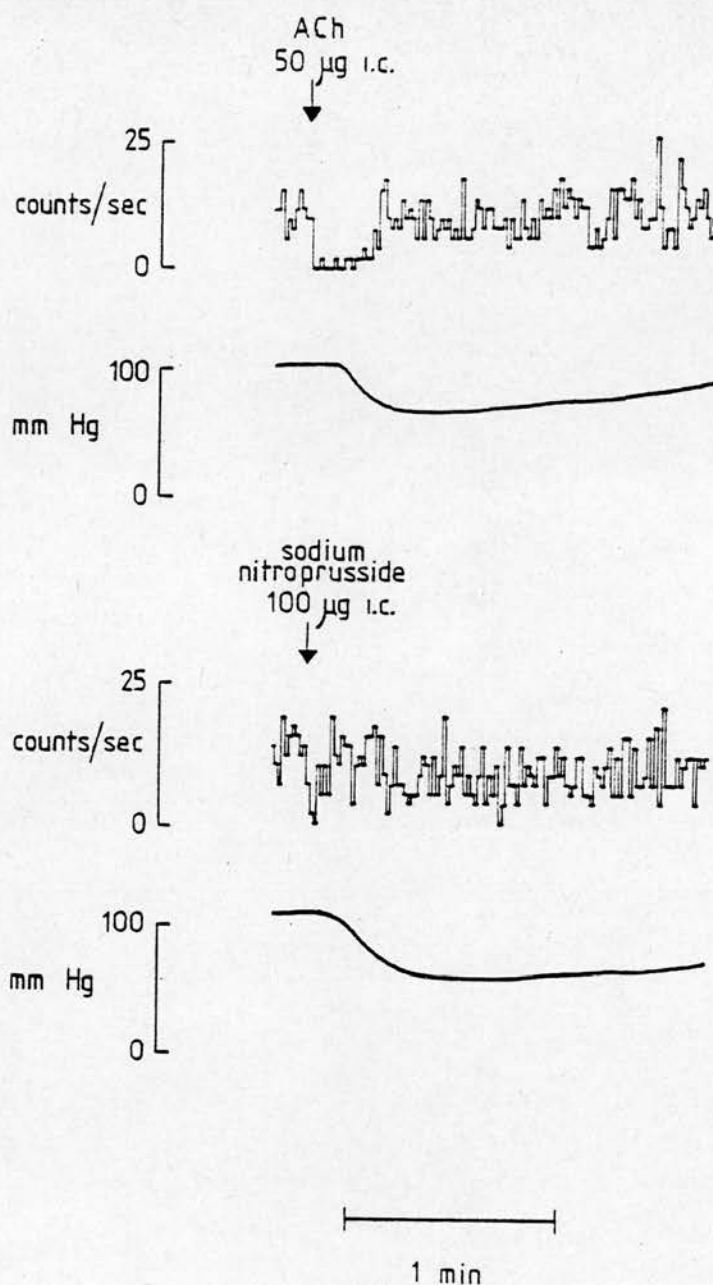


Figure 4.18

Response of carotid chemoreceptors (three units) to ACh and sodium nitroprusside. Panels show from above downwards : discharge frequency and mean B.P. Injections were made at the arrows. The time bar refers to both panels.

or caused a slight inhibition of chemoreceptor activity. The magnitude of the inhibition did not appear to be dose-related and was only slightly greater than that produced by an equal volume (0.1 ml) of Locke solution. The effectiveness of sodium nitrite as a vasodilator in the rabbit is uncertain, however, since injection of sodium nitrite had very little effect on arterial B.P.

## 2. Sodium nitroprusside

Sodium nitroprusside, like sodium nitrite, is a potent vasodilator which acts directly on vascular smooth muscle (Johnson, 1929). Injection of sodium nitroprusside (10 - 50  $\mu$ g I.C.) caused a short-lasting (4 - 7 sec) inhibition of chemoreceptor activity, which did not appear to be dose-related, and evoked a marked depressor response (see Figure 4.23). In some experiments sodium nitroprusside caused a delayed increase in chemoreceptor activity which was dose-dependent.

## DISCUSSION

Although the first reported recordings of afferent impulse activity in the sinus nerve were obtained using rabbits as the experimental animal (see Bronk and Stella, 1932) there have, since that time, been few electrophysiological studies of afferent chemoreceptor activity in this species - cats or, less frequently, dogs being the preferred species for such experiments. It is therefore worthwhile to briefly discuss the advantages and disadvantages of using rabbits as experimental animals in this type of study. The greatest advantage is that of cost, since rabbits, for experimental purposes, may be obtained more cheaply than cats or dogs. A second



advantage is that the sinus nerve of the rabbit is not embedded in muscle or connective tissue as is the corresponding nerve in the cat or dog and is consequently more easily accessible by surgery. These advantages are, however, offset by the disadvantage that a low success rate, at least for recording chemoreceptor activity, may be expected and hence a larger number of animals and, perhaps more important, a greater amount of time must be expended in order to obtain useful data. This disadvantage does not apply to experiments in which it is desirable to study afferent baroreceptor activity which is relatively easy to record from the rabbit sinus nerve (see Appendix I) making this species particularly suitable for such a study. Rabbits were chosen as experimental animals in the present investigation partly for financial reasons and partly due to the fact that a study of the effects of ACh, DA and 5-HT on afferent chemoreceptor activity in this species had not previously been performed.

The results show that ACh and DA both inhibit chemoreceptor activity while 5-HT causes excitation followed by inhibition of activity in anaesthetized rabbits. The responses of the rabbit carotid chemoreceptors to DA and 5-HT were qualitatively similar to the corresponding responses of cat carotid chemoreceptors (see Section III). The inhibitory response to ACh, however, was unexpected, being in complete contrast to the stimulant action obtained in other species (see Section I). Experiments were performed to investigate the mechanism whereby ACh inhibits chemoreceptor activity. The results obtained are discussed below.

### Vascular effects

Sampson and Vidruk (1977) found that ACh caused depolarisation of the d.c. potential recorded from the sinus nerve of the rabbit carotid body *in vitro*. Since this response is characteristic of substances which stimulate chemoreceptor discharge in this preparation, e.g. NaCN, the possibility that the inhibition of chemoreceptor activity produced by ACh in the rabbit carotid body *in situ* was due to a vascular effect must be considered. Inhibition of chemoreceptor discharge could have resulted from an action of ACh on vascular smooth muscle, although the short latency to onset of the effect argues against this, at least for the early part of the response. Vasodilatation of the carotid body vasculature would increase blood flow into the glomus and might thereby be expected to reduce spontaneous chemoreceptor discharge before any tendency for discharge to increase secondary to the delayed fall in B.P. caused by circulation of the ACh, although autoregulation of blood flow which can occur in the rabbit carotid body (McCloskey, 1968) complicates this speculation. It was found that administration of doses of atropine (5 or 10 mg/kg I.V.) sufficient to block the ACh-induced fall in systemic B.P. reduced, but did not abolish, the chemoreceptor response to ACh. The vascular explanation therefore seems unlikely, providing that the carotid body vasculature responsiveness to ACh is similar to that of the peripheral vasculature. This may or may not be the case. Also, it is possible that the high local concentration of ACh following I.C. injection was sufficient to overcome the antagonistic effect of atropine in the region of the carotid sinus. It was found, however, that I.C. administration of the vasodilators sodium nitrite and sodium nitroprusside had little

effect on chemoreceptor activity. It is questionable whether sodium nitrite is an effective vasodilator agent in rabbits in the doses used in the present study since administration of this substance had very little effect on systemic B.P. Sodium nitroprusside, however, caused a marked fall in B.P., presumably due to its vasodilator action, but had little effect on chemoreceptor activity. The delayed increase in discharge observed following administration of sodium nitroprusside in some experiments was probably due to production of cyanide ions during the conversion of nitroprusside to thiocyanate. These results with atropine and sodium nitroprusside indicate that although vasodilatation may explain a small part of the inhibition of chemoreceptor activity evoked by ACh, it is not responsible for the greater part of the effect. This view is supported by the recent finding that ACh inhibits afferent chemoreceptor activity in the superfused rabbit carotid body *in vitro* (Monti-Bloch and Eyzaguirre, 1977). In view of these observations it seems doubtful whether results obtained by measuring d.c.potential changes in the sinus nerve, such as those of Sampson and Vidruk (above), may be compared meaningfully with results obtained by measuring afferent chemoreceptor activity.

Very recently, Matsumoto, Nagao, Ibi and Nakajima (1979) found that I.C. injections of ACh evoked reflex bradycardia in rabbits, an effect which the authors assumed was due to stimulation, by ACh, of carotid chemoreceptors. The authors did not, however, check to see whether this response was abolished by section of the sinus nerve. In the light of the present results it seems unlikely that the reflex bradycardia observed by these workers was due to stimulation of carotid chemoreceptors. In fact, the opposite result

would be expected, i.e. ACh, by inhibiting afferent chemoreceptor activity, would be expected, if anything, to cause a slight tachycardia by transiently depressing any tonic inhibitory influence of afferent chemoreceptor activity on heart rate. It is not clear, however, whether a short-lasting depression of chemoreceptor activity in rabbits, such as that produced by I.C. injection of ACh, is reflected in reflex adjustments of heart rate, vasomotor tone or respiration. Results obtained in this laboratory (A. Morris, unpublished observations) suggest that this is not the case and Wiemer (1962) has reported that the carotid chemoreceptors do not participate in the respiratory response to I.V. injections of ACh in rabbits. The bradycardia observed by Matsumoto *et al* (above) following I.C. injection of ACh was probably due to an action of ACh on the C.N.S.

#### Characterization of the cholinergic receptors

Experiments were performed in an attempt to identify the cholinergic receptors responsible for the effects of ACh on chemosensory activity. High doses of ACh caused a transient stimulation of the chemoreceptors in some experiments, an effect which preceded the inhibition. This excitation appeared to be attributable to a nicotinic action of ACh since it was blocked by mecamylamine. Monti-Bloch and Eyzaguirre (1977) found that nicotine stimulated the chemoreceptors in the rabbit carotid body *in vitro*, an effect which was blocked by D-TC. In the present experiments SDCh was used as a nicotinic agonist rather than nicotine since it has been reported that in cats, repeated administration of the latter substance leads to blockade of the nicotinic receptor whereas SDCh evokes consistent excitatory chemoreceptor responses without develop-



ment of tachyphylaxis (Anichkov and Belen'kii, 1963; Dardymov and Ger, 1964). It was found, however, that SDCh had no consistent effect on chemoreceptor activity in the rabbit. Since the excitatory effect of ACh was mediated by a nicotinic mechanism, one would expect SDCh to evoke a similar, albeit slight, excitatory response. It is not clear why this did not occur. In any event, since the excitatory effect of ACh was slight and was only observed following injections of high doses, it is unlikely to have much physiological significance.

The inhibitory action of ACh was evidently mediated by a muscarinic mechanism since the muscarinic agonists BCh and MCh also caused inhibition of chemoreceptor activity. SDCh sometimes evoked a slight inhibition of chemoreceptor activity but this was probably due to a weak action on muscarinic receptors. ACh-induced inhibition of chemoreceptor activity was blocked by administration of the muscarinic antagonist atropine, though relatively high doses were needed. Interpretation of data derived from experiments involving the use of atropine in rabbits is complicated by the presence of an atropinase enzyme in some rabbits (Ambache, 1955), a fact which might account for the large variation in the dose of atropine required to block the vasodepressor action of ACh. This might also be the reason why high doses of atropine were required to block ACh- or BCh-induced inhibition of chemoreceptor activity. Alternatively, it may be that the muscarinic receptors mediating the inhibition of chemoreceptor activity are 'atypical', perhaps being similar to those in the adrenal medulla (Henderson and Ungar, 1977), or in sympathetic ganglia (Hilton, 1977), which are also fairly resistant to atropine blockade. A better understanding of the mechanism by



which ACh inhibits chemoreceptor activity in the rabbit might be gained by studying the effects of a non-esteratic muscarinic blocking agent of similar potency to atropine, e.g. tricyclamol (Barlowe, 1955), on the responsiveness of rabbit carotid chemoreceptors to ACh since such a drug would be resistant to the action of atropinase (atropine esterase). Unfortunately, it was not possible to perform this experiment during the present investigation.

#### ACh-DA interaction

It has been suggested that ACh may release DA in the rat carotid body via a muscarinic mechanism (Hellström, Hanbauer and Costa, 1976). Since it was found that I.C. injection of DA caused inhibition of rabbit chemosensory activity, and DA is present in the rabbit carotid body (Dearnaley *et al*, 1968), the possibility that ACh-induced inhibition of chemoreceptor activity was secondary to DA release became attractive. This is unlikely to be the case, however, since  $\alpha$ -flupenthixol, in doses sufficient to block the inhibitory response to exogenous DA, did not reduce the inhibitory response evoked by ACh.

Although it was found in the present experiments that injections of DA inhibit chemoreceptor activity in the rabbit, the response being similar to that observed in cats (see Section III), it should be noted that Monti-Bloch and Eyzaguirre (1977) reported that DA increases chemoreceptor discharge in the superfused rabbit carotid body *in vitro*. A possible explanation for the difference between the present results, obtained in the carotid body *in situ*, and those obtained in the *in vitro* preparation is that vascular effects are responsible for the inhibition observed *in situ*; such vascular changes should not affect activity in the superfused

preparation. However, vascular effects are unlikely to account for DA-induced inhibition of chemoreceptor activity in cats (see Section III) and by analogy it is unlikely that inhibition of chemoreceptor activity in rabbits is secondary to vascular effects of DA, particularly since the latency to onset of the effect is so short (< 2 sec). Zapata (1975) found that DA produced excitation of chemoreceptor activity in the cat carotid body *in vitro* following desensitization of DA inhibitory receptors (see Section I). It is possible that the excitatory chemoreceptor response to DA in the rabbit carotid body *in vitro*, observed by Monti-Bloch and Eyzaguirre, was due to desensitization of DA inhibitory receptors. It was found in the present experiments that, on certain occasions, DA produced a delayed excitation which followed the inhibitory effect. Also, DA tended to cause excitation of chemoreceptor activity following administration of  $\alpha$ -flupenthixol. It may be that there are both inhibitory and excitatory receptors for DA in the rabbit carotid body and that endogenous DA in this species has a similar physiological role to that suggested for cats in Section III. Injection of D-amphetamine (10  $\mu$ g I.C.) caused inhibition of chemoreceptor activity, an effect which was blocked by  $\alpha$ -flupenthixol. In terms of the original rationale for using amphetamine, i.e. to release DA (see Section III), this result could be interpreted as suggesting that endogenous DA has an inhibitory action on chemoreceptor activity. It was found in cats, however, that although low doses of amphetamine inhibit chemoreceptor activity, high doses cause stimulation of activity (see Section III). It may be that in rabbits, as in cats, inhibition of chemoreceptor activity produced by low doses of amphetamine is due to an agonist action on DA inhibitory receptors

rather than secondary to release of endogenous DA. It would be interesting to determine the effects of high doses of amphetamine on chemosensory activity in the rabbit and to study the effects of DA-uptake blockers on responses to chemoreceptor stimulants to see if such treatments have a similar effect on chemosensory activity in rabbits as in cats. The response of the rabbit carotid chemoreceptors to NaCN was potentiated by  $\alpha$ -flupenthixol, which supports the idea that endogenous DA is released during chemoreceptor stimulation and has an inhibitory effect on afferent chemoreceptor activity. Whether endogenous DA also has an excitatory effect on chemoreceptor activity in this species remains to be established but the similarities in the responsiveness of the rabbit carotid chemoreceptors to DA to that of the cat carotid chemoreceptors suggests that this might be the case.

The inhibitory response to DA was slightly reduced by a high dose of atropine (1 mg/kg I.C.) and the duration of the response was increased following administration of physostigmine (0.1 - 0.25 mg/kg I.C.). This might be taken to mean that DA-induced inhibition of chemoreceptor activity was secondary to ACh release. It is possible, however, that the effects of physostigmine on the response to DA were brought about indirectly by flow changes arising from enhanced cholinergic transmission in the superior cervical ganglion, since the ganglio-glomerular nerve was intact during experiments with this anticholinesterase drug. This might also explain the variability of responses to NaCN following administration of physostigmine. The reduced inhibitory response to DA following administration of atropine may have been due to a non-specific action of the high dose of atropine required to produce this effect.

Nevertheless, on the basis of the above results, the possibility that DA causes release of ACh in the rabbit carotid body cannot be precluded. The present experiments were designed primarily to study the inhibitory effects of ACh on rabbit carotid chemoreceptor activity and most of the data relating to the effects of cholinergic antagonists on responses to DA are of a qualitative rather than a quantitative nature. A quantitative study of the effects of cholinergic antagonists on responses to DA is required to clarify this point.

The response of the rabbit chemoreceptors to 5-HT was very similar to the response of the cat carotid chemoreceptors to this substance (see Section III). It is unlikely that either the excitatory or the inhibitory effects of 5-HT were secondary to release of ACh since responses to 5-HT were not appreciably affected by cholinergic blocking agents. The possibility that ACh might release 5-HT in the rabbit carotid body was not investigated in the present study.

#### Physiological significance

It is difficult to reconcile the present finding that ACh inhibits rabbit chemosensory activity with the hypothesis that ACh is an *excitatory* transmitter in the chemosensory mechanism (see Section I). The nicotinic action of ACh in exciting chemoreceptor nerve endings in cats and dogs may be non-specific, i.e. a direct action on the unmyelinated, terminal portion of the sensory nerve which can be blocked pharmacologically without affecting the response to physiological stimuli (see Section I). It could be that whereas non-specific excitation of chemosensory afferents is observed in the cat and dog, it is not obtained, except transiently following



high doses of ACh, in the rabbit, i.e. the threshold for non-specific excitation by ACh is higher in the rabbit than in cats or dogs. If this is the case, and ACh has nothing to do with transmission of afferent chemosensory impulses, it is difficult to explain why mecamylamine reduced chemoreceptor responses to NaCN and hypoxia. It could be argued that any tendency for injected ACh to cause stimulation of afferent activity in rabbits is masked by the inhibitory effects of the drug. The arrangement of cholinergic receptors on the sensory nerve end may be such that the inhibition-mediating muscarinic receptors are more accessible to injected ACh than the excitation-mediating nicotinic receptors while the nicotinic receptors are more accessible to endogenously released ACh than the muscarinic receptors. In this scheme mecamylamine might be expected to reduce the chemoreceptor response to NaCN or hypoxia by antagonising the excitatory action of endogenously released ACh even though the response to injected ACh was inhibitory. This argument is very similar to that proposed in Section III to explain the effects of DA and drugs which modify dopaminergic systems on chemoreceptor activity in the cat. Whereas in the cat and rabbit DA tended to produce excitatory effects when the inhibitory effects of DA were blocked, however, ACh did not produce excitatory responses in the rabbit when its inhibitory action was blocked by atropine. Further, physostigmine, which would be expected to enhance the effects of endogenously released ACh, did not increase the magnitude of chemoreceptor responses to NaCN and had little effect on responses to hypoxia. It seems unlikely, therefore, that endogenous ACh has an excitatory effect on afferent chemoreceptor activity. The suggestion that ACh-induced excitation of chemoreceptor activity



is due to a non-specific action of the drug and that this effect has a high threshold in the rabbit (above) seems a more appropriate explanation of results. The inhibitory effect of mecamylamine on chemoreceptor responses to NaCN and hypoxia is unlikely to be due to the nicotinic receptor blocking properties of the drug but may be due to a non-specific action. This possibility is explored further in Section V.

The question must now be asked what role, if any, does ACh play in the chemosensory mechanism? It may well be that the inhibition evoked by ACh in rabbits is either indirectly mediated or else non-specific and lacking physiological significance, but it seems worth exploring the possibility that these findings with exogenous ACh in rabbits, free from the masking excitatory effects of the drug, provide a clue regarding the physiological role of ACh in the carotid body. If endogenous ACh has an inhibitory action on afferent chemoreceptor activity, physostigmine, which enhanced the inhibitory response to injected ACh, might be expected to reduce responses to chemoreceptor stimuli and high doses of atropine, which blocked the inhibitory response, might be expected to cause potentiation of responses to stimulants. This was not found to be the case, in fact, responses to NaCN were reduced following administration of high doses of atropine. It is unlikely, therefore, that ACh is important to an intrinsic mechanism of control of chemoreceptor discharge frequency. There is, however, the possibility that ACh is important to an extrinsic control mechanism. In all the experiments performed in the present study, the sinus nerve was cut in order to facilitate dissection of the nerve and obtain recordings of afferent activity. This procedure, however, obviates the influence of any descending,

i.e. efferent pathway which might travel in the sinus nerve. There is evidence for an efferent pathway in the sinus nerve to the cat carotid body (Biscoe and Sampson, 1968) and stimulation of this pathway inhibits chemosensory activity (Neil and O'Regan, 1969; Belmonte and Eyzaguirre, 1974). It has been shown that efferent inhibition can be reduced by administration of atropine (Goodman, 1975; Willshaw, 1976; Sampson, Aminoff, Jaffe and Vidruk, 1976b) or  $\alpha$ -adrenergic blocking agents (Sampson, 1975; Sampson, Aminoff, Jaffe and Vidruk, 1976b). It has been proposed that the efferent pathway is part of an inhibitory feedback system which involves release of ACh, or some other substance with muscarine-like properties, from efferent nerve ends which acts on type I cells to release catecholamines which in turn mediate the inhibitory effect by an  $\alpha$ -adrenergic mechanism (Sampson, 1972). It is not known whether the rabbit carotid body receives an efferent innervation. Nevertheless, the possibility exists that the inhibitory effect of ACh on chemosensory activity in rabbits might result from an action on muscarinic receptors involved in such a pathway. On the basis of the present results it seems unlikely that the inhibitory effect of ACh is secondary to DA release but the possibility that the effect is secondary to release of NA or ADR cannot be precluded since the effects of these substances on chemoreceptor activity in this species have not been studied. In this scheme ACh would be a transmitter at efferent nerve endings in the carotid body, acting either directly or through release of another substance(s) which modulates or regulates chemoreceptor activity rather than an excitatory transmitter at afferent synapses between type I cells and sensory nerve endings.

## SECTION V

### GENERAL DISCUSSION AND CONCLUSIONS

The results presented in this thesis suggest two important conclusions,

- a) endogenous DA may have an excitatory effect on chemoreceptor activity in the mammalian carotid body, and
- b) ACh is unlikely to act as an excitatory transmitter at carotid chemoreceptors but might act as a transmitter in an inhibitory efferent pathway.

The possibility that DA has an excitatory effect on carotid chemoreceptor activity in the cat even though, under normal circumstances, injected DA evokes inhibition of activity may explain the apparent species differences in the response to injected DA observed in cats and rabbits and that observed in dogs (see Section I). The different responses to injected DA, i.e. excitation in dogs and inhibition in cats and rabbits, may, as Lladós and Zapata (1978a) suggest, be due to a difference in the accessibility of the excitatory receptors to injected DA. Very recently, Bisgard, Mitchell and Herbert (1979) showed that I.C. injection of low doses of DA (usually  $<10 \mu\text{g}$ ) inhibited carotid chemoreceptor activity in dogs and that DA produced an excitatory response, such as that observed by Black *et al* (1972) in this species, only when higher doses of DA ( $\geq 10 \mu\text{g}$  I.C.) were administered. Bisgard *et al* also found that the inhibitory effects of DA in dogs were blocked by haloperidol which suggests that the receptor mediating chemosensory inhibition in the dog is the same or a similar type to that mediating chemosensory inhibition in the cat and rabbit. It seems that injection of DA can cause inhibition and/or excitation in cats and dogs (and probably also in rabbits) depending on the dose of DA administered. Under

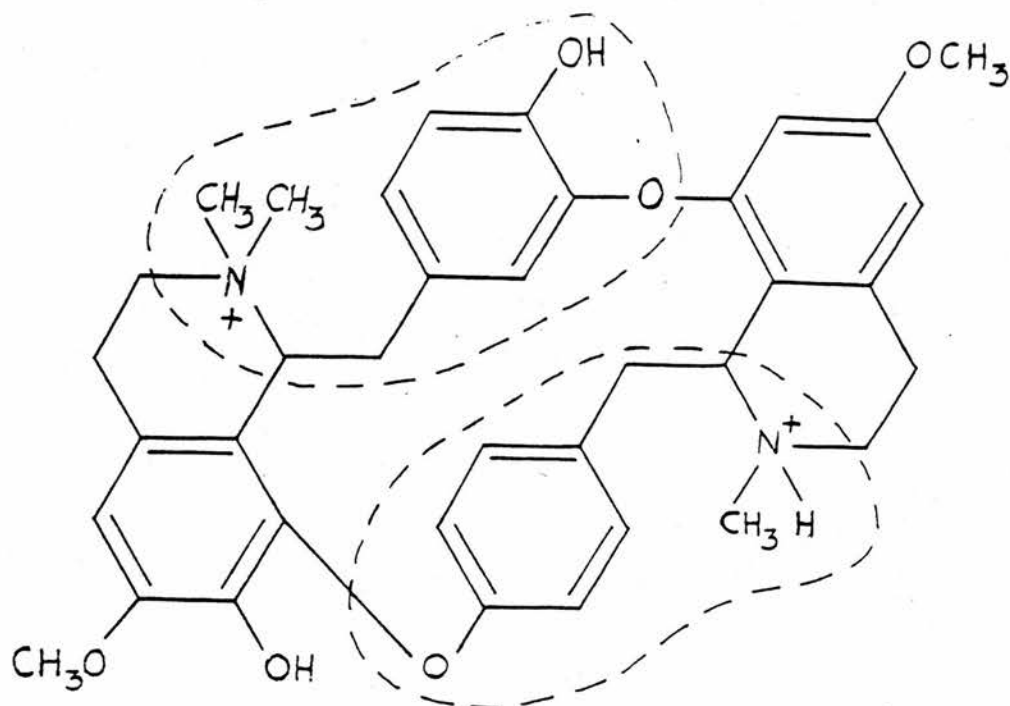
normal circumstances, the excitatory effect of DA on chemoreceptor activity in the cat is delayed and is preceded by inhibition (Zapata, 1975, 1977; Nishi, 1977; Llados and Zapata, 1978a; Zapata and Llados, 1978; see also Section III of this thesis). According to Bisgard *et al*, the opposite is true in dogs, i.e. DA causes excitation followed by inhibition. This may be due to a difference in the accessibility to injected DA of excitatory and inhibitory receptors.

There is evidence that at least two types of DA receptor are present in the mammalian C.N.S., one of which is linked to the enzyme adenylate cyclase (designated D1) and the other (designated D2) which is not (see Keabian and Calne, 1979). The pharmacological characteristics of the receptor mediating the inhibitory effects of DA on carotid chemoreceptor activity, i.e. stimulation by apomorphine and inhibition by  $\alpha$ -flupenthixol or haloperidol, seem similar to the non-adenylate cyclase linked receptor found in the C.N.S. and may therefore be of the same or a similar type to the D2 receptor (Keabian and Calne, 1979). It is tempting to associate the excitation-mediating receptor in the carotid body with the D1 receptor, especially since cAMP stimulates afferent chemoreceptor activity in cats (Joels and Neil, 1968).  $\alpha$ -Flupenthixol antagonises, although not specifically, the D1 receptor in the C.N.S. (see Keabian and Calne, 1979), however, and this is clearly not the case for the DA receptor mediating chemoreceptor stimulation in cats and rabbits. Nevertheless it might be interesting to study the effects of phosphodiesterase (which metabolises cAMP) inhibitors on chemoreceptor activity and the effects of cholera toxin which is a non-specific activator of adenylate cyclase (see Gill, 1977).



The excitatory effect of DA on chemoreceptor activity in the dog was blocked by D-TC but not by gallamine (Bisgard *et al*, 1979). The receptor mediating this effect might be similar therefore to the receptor mediating DA-induced depolarization of neurone in the molluscan nervous system which is also blocked by D-TC (Ascher, 1972). LSD is a specific ligand for the depolarization-mediating DA receptor in the mollusc (Drummond, Bucher and Levitan, 1978) and Nishi (1975) has found that LSD stimulates carotid chemoreceptor activity in the cat carotid body *in vivo*. Nishi offered no explanation for the effect of LSD on chemoreceptor activity (see Section I) but it may be that this effect was due to an action of LSD on excitation-mediating DA receptors. These results cannot be considered as direct evidence that the receptor mediating the excitatory effect of DA on chemosensory activity in cats and dogs (and probably also in rabbits) and the receptor mediating depolarization of neurone in the molluscan nervous system are of the same type but they are certainly suggestive. It is not known whether D-TC blocks the excitatory effects of DA on chemoreceptor activity in cats and rabbits. It has been found, however, that D-TC inhibits chemoreceptor activity in the cat carotid body *in vitro* (Eyzaguirre and Koyano, 1965b) and this has been considered as evidence in favour of the 'ACh hypothesis' of carotid chemoreception (see Section I). It is possible, however, that this effect of D-TC was due to blockade of DA excitatory receptors rather than cholinergic receptors. Indeed, in view of the fact that ACh is unlikely to act as an excitatory transmitter in the carotid body (see Section IV), this seems a more probable explanation. It is not entirely surprising that D-TC should act as an antagonist at DA receptors since the chemical structure of D-TC is, in some

# D-TC



# DA

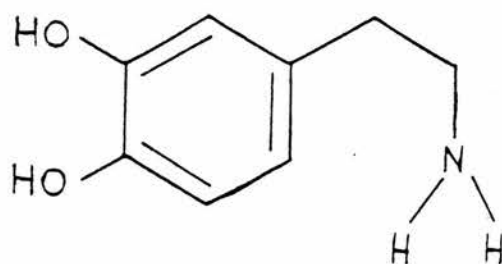


Figure 5.1

Schematic diagrams of the molecular structure of D-TC and DA. The regions of the D-TC molecule enclosed in the dotted lines can be seen to be structurally similar to DA.

respects, similar to that of DA (see Figure 5.1). It was found in the present study that mecamylamine slightly inhibited the response of rabbit carotid chemoreceptors to NaCN and hypoxia even though, in this species, ACh itself causes inhibition of chemoreceptor activity. Those authors who reported that mecamylamine inhibits responses of cat carotid chemoreceptors to NaCN and hypoxia found that much higher doses of mecamylamine were required to produce this effect than to block responses to ACh (see Section I) which might be taken to indicate that the effect was unlikely to be due to the nicotinic blocking action of the drug. As well as blocking cholinergic nicotinic receptors, however, mecamylamine inhibits release of DA from neurons in the C.N.S. (Ahtee and Kaakkola, 1978). It is possible that this drug inhibits release of DA from type I cells in the carotid body. It could be that some drugs, better known for their effects on cholinergic systems, bring about their effects on chemoreceptor activity by non-specific actions on a dopaminergic mechanism. This suggestion, though speculative, could help to explain some of the anomalies in the literature regarding the effects of cholinergic agonists and antagonists on carotid chemoreceptor activity. It would be interesting to determine whether D-TC blocked the excitatory effects of DA in cats and rabbits. This could be done by studying the effects of D-TC on responses to DA in animals pretreated with haloperidol or  $\alpha$ -flupenthixol. It would also be interesting to determine whether D-TC or mecamylamine reverse the potentiating effects of DA-uptake blockers on responses to chemoreceptor stimulants. The possibility that D-TC is an antagonist at a novel DA receptor, i.e. the chemoreceptor excitation/molluscan neuron depolarization mediating receptor, could have interesting

implications in terms of the development of new drugs effective as agonists or antagonists at this receptor.

In view of the evidence presented in Section IV it seems highly unlikely that ACh is an excitatory transmitter of sensory impulses in the carotid body. The excitatory effect of ACh on chemosensory activity in cats and dogs appears to be due to a non-specific action of ACh on sensory nerve ends, an interpretation which agrees well with results presented in Section III where it was found that ACh-induced stimulation of chemoreceptor activity in the cat is not appreciably affected by  $\alpha$ -flupenthixol (except at high doses) or nomifensine, and is slightly reduced by benztropine even though responses to NaCN and hypoxia were potentiated by these substances. The most likely physiological role for ACh in the carotid body is as a transmitter in an inhibitory efferent pathway (see Section IV). The mechanism of efferent inhibition in cats appears to have two components - a vascular and a non-vascular component (O'Regan, 1977). Increased efferent activity in the sinus nerve in cats causes increased glomerular blood flow and, as a consequence, afferent chemoreceptor activity is reduced (Neil and O'Regan, 1969, 1971). This is the vascular component and a number of authors are of the opinion that this is the sole mechanism of efferent inhibition (Goodman, 1973; Belmonte and Eyzaguirre, 1974; McCloskey, 1975). There is, however, evidence that efferent inhibition may be demonstrated during total abolition of carotid body blood flow (O'Regan, 1974) indicating that a non-vascular component also exists though this is probably less important physiologically, at least in cats, than the vascular component (O'Regan, 1977). ACh-induced inhibition of chemoreceptor activity in rabbits did not appear to be mediated

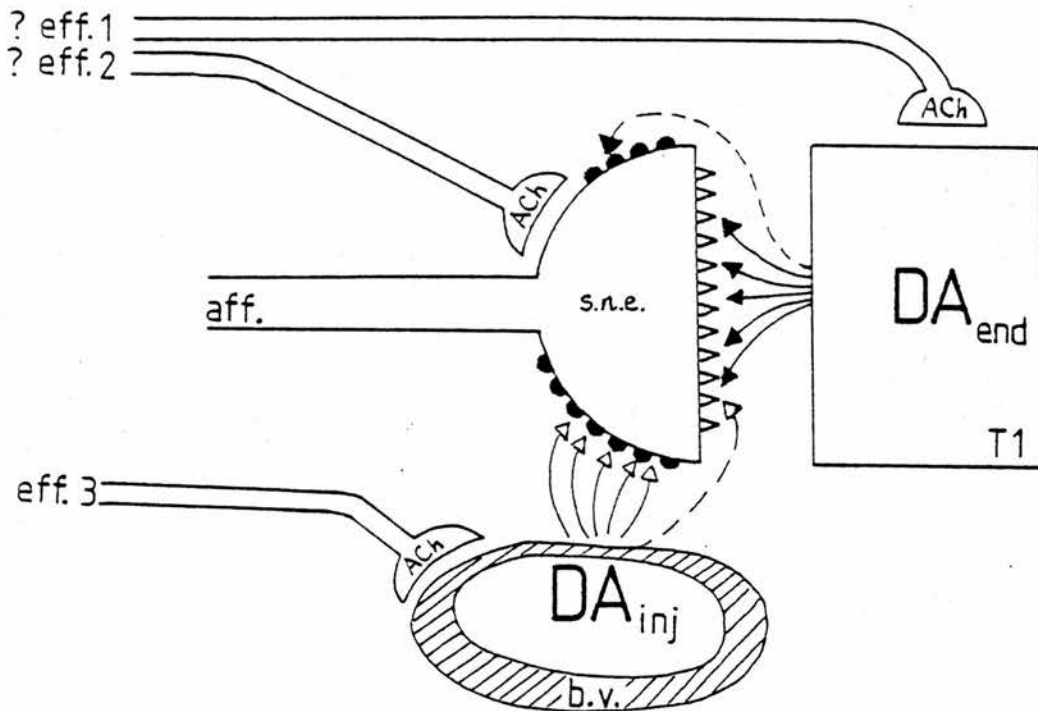
by a vascular mechanism (see Section IV). If the inhibitory action of ACh was due to an action on muscarinic receptors involved in an efferent inhibitory pathway then this would suggest that efferent inhibition in the rabbit has a strong non-vascular component. Rabbits, therefore, might prove to be useful experimental animals for studying non-vascular mechanisms of efferent inhibition. This is pure speculation, of course, since the inhibitory effect of ACh on rabbit chemosensory activity may have nothing to do with efferent inhibition. It must be established, by recording from the central cut end of the sinus nerve, whether the rabbit carotid body receives an efferent innervation and if so, what influence this pathway has on chemoreceptor activity before any correlation between the inhibitory effects of ACh on chemosensory activity and efferent inhibitory mechanisms may be made.

The possibility that DA acts as a sensory transmitter in the carotid body must now be considered, i.e. that the chemoreceptor elements in the carotid body are presynaptic to sensory nerve endings and increased afferent activity occurs as a consequence of increased DA release from type I cells in response to chemoreceptor stimuli. In favour of this idea is evidence which suggests that the carotid body stores DA which is released in response to chemoreceptor stimuli (see Section I). Also, the results presented in this thesis suggest that endogenous DA has an excitatory effect on carotid chemoreceptor activity. Studies of the effects of pharmacological depletion of catecholamines, by reserpine or 6-OHDA, on carotid chemoreceptor activity have produced equivocal results (see Section I). Zapata *et al* (1969) obtained recordings of carotid chemoreceptor activity in cats following chronic exposure of the animals to reserpine.



Under these conditions the DA content of the carotid body was reduced by up to 90 per cent. Without knowing what percentage of the total amount of DA stored in the carotid body is required to maintain normal function, however, few conclusions can be drawn from this result. Antonaccio and Smith (1974) found that the response of atrial muscle to sympathetic nerve stimulation was unimpaired following 90 per cent depletion of NA stores by reserpine. Conceivably, 10 per cent of the total DA content of the carotid body could be sufficient to maintain its function as a sensory transmitter. There are, however, a number of reasons why it is unlikely that DA is an excitatory transmitter of afferent chemoreceptor activity. For the dopaminergic mechanism described above to operate, sensory nerve ends must be apposed to type I cells. It has been found, however, that chemoreceptor activity may be recorded from regenerated axons of the sinus nerve which terminate in neuromas containing no type I cells (Mitchell, Sinha and McDonald, 1972; Mitchell and McDonald, 1975) or from regenerating afferent fibres which are not in contact with type I cells (Bingmann, Kienecker and Knoche, 1977) although other authors have found that afferent fibres must be in contact with type I cells before afferent activity may be recorded (Verna, Roumy and Leitner, 1975; Zapata, Stensaas and Eyzaguirre, 1969). The carotid body may be functionally reinnervated with vagal (De Castro, 1951) or superior laryngeal nerve fibres (Zapata, Hess and Eyzaguirre, 1969) which does not support the case for DA as a sensory transmitter unless it is assumed that vagal and superior laryngeal nerve endings have excitatory DA receptors, an assumption for which there is no evidence whatsoever. In any case, doses of D-TC sufficient to abolish the excitatory

effects of injected DA on chemosensory activity in the dog reduce, but do not abolish, chemoreceptor responses to NaCN and hypoxia (Bisgard et al, 1979) which suggests that although endogenous DA may have a stimulant effect on chemoreceptor activity *in vivo*, this is not the sole mechanism by which afferent chemoreceptor discharge is initiated. It may be that the sensory nerve ends are themselves the chemoreceptors, as Biscoe (1971) suggests, and that DA modulates activity in the nerve endings. A model for the relationship between sensory nerve endings and type I cells, based on this idea, would be similar to that proposed by McDonald and Mitchell (1975) (see Section 1) save that instead of type I cells acting as inhibitory interneurons, the present results suggest that the type I cells act as excitatory interneurons, i.e. as endogenous 'amplifiers' of afferent chemoreceptor activity. In this scheme increased activity in the sensory nerve would be accompanied by increased release of DA. The DA then acts on excitatory receptors on the sensory nerve ending to boost activity. During strong chemoreceptor stimulation, e.g. in response to NaCN, large amounts of DA are released, some of which flows out of the synapse and acts on inhibitory receptors which are located on the sensory nerve ending on the periphery of the synaptic region. Activation of inhibitory receptors in this manner limits the excitatory effects of DA. DA release would be in response to a direct effect of chemoreceptor stimulants on type I cells or secondary to release of an unidentified substance, itself released from the sensory nerve endings. This scheme has the physiological advantage that, for a stimulus of a given strength, the frequency of discharge of individual chemoreceptor units could be controlled by the relative numbers of excitatory and inhibitory receptors for DA



- = major action } DA<sub>end</sub>
- - -→ = minor action } DA<sub>end</sub>
- = major action } DA<sub>inj</sub>
- - -→ = minor action } DA<sub>inj</sub>
- Δ = excitatory DA receptor
- = inhibitory DA receptor

Figure 5.2

Schematic diagram of proposed control mechanisms of afferent chemoreceptor discharge. Most of the endogenous DA (DA<sub>end</sub>) released acts (—→) at excitatory DA receptors but some acts (- - -→) at inhibitory receptors. Injected DA (DA<sub>inj</sub>) acts (—→) mainly at inhibitory receptors but some may act (- - -→) at excitatory receptors. Possible efferent pathways (eff.1, 2 and 3) are also included in the diagram. Eff.1 and eff.2 represent possible non-vascular pathways in which ACh acts either on the sensory nerve end (s.n.e.) or type I cell (T1) and eff.3 represents the vascular pathway - release of ACh causing vasodilatation and consequently increased carotid body blood flow.

on sensory nerve endings which would allow a measure of differentiation between the chemoreceptors at the peripheral level. A schematic diagram illustrating the mechanism described above is given in Figure 5.2. Figure 5.2 also includes representations, based on the suggestions of O'Regan (1977), of possible interactions of a cholinergic, non-vascular, inhibitory efferent pathway with the proposed dopaminergic mechanism.

Research in the field of carotid chemoreceptors has placed considerable emphasis on the chemoreceptor stimulant effects of ACh and, especially in recent years, the inhibitory effects of DA. On the basis of the results obtained in the present study it is concluded that although these effects may be important, the opposite effects, i.e. inhibition in the case of ACh and excitation in the case of DA, are of greater importance to the chemoreceptive mechanism *in vivo*. Perhaps a re-evaluation of data derived from experiments designed to study the carotid body chemoreceptors, especially those involving the use of pharmacological substances, in these terms may be of value in reaching a better understanding of the mechanism by which chemoreceptor activity is initiated.

APPENDIX I

AFFERENT ACTIVITY IN THE RABBIT SINUS NERVE



## 1. Chemoreceptor activity

Recordings of chemoreceptor activity were obtained from the sinus nerves of only about 30 per cent of the rabbits used in the present study. It is unlikely that this low success rate was due to surgical incompetence since recordings of chemoreceptor activity were obtained in about 90 per cent of experiments on cats in which similar surgical procedures were used. The criteria adopted to identify afferent chemoreceptor activity,

- a) random frequency of discharge,
- b) increased activity in response to NaCN,
- c) increased activity in response to hypoxia, and
- d) decreased activity in response to hyperoxia,

were the same for both cats and rabbits and seem adequate. No recordings were obtained which satisfied criteria (c) and (d) which did not also satisfy (a) and (b). Changing the strain of rabbits or the anaesthetic used in the experiments did not increase the success rate in obtaining recordings of chemoreceptor activity. Two possible explanations for the low success rate are, firstly, that due to a peculiarity of carotid body chemoreceptors in some rabbits, the sinus nerve must be intact for normal function or, secondly, the fibres conducting afferent chemoreceptor activity in rabbits are more fragile than the corresponding fibres in cats and are therefore more liable to be damaged during fine dissection of the nerve. The first possibility seems unlikely. The second possibility is feasible, however, since it has been shown that afferent chemoreceptor activity in the rabbit sinus nerve is conveyed by slow-conducting, small diameter ( $< 2 \mu\text{m}$ ) myelinated fibres (Laurent and Barrés, 1964). The

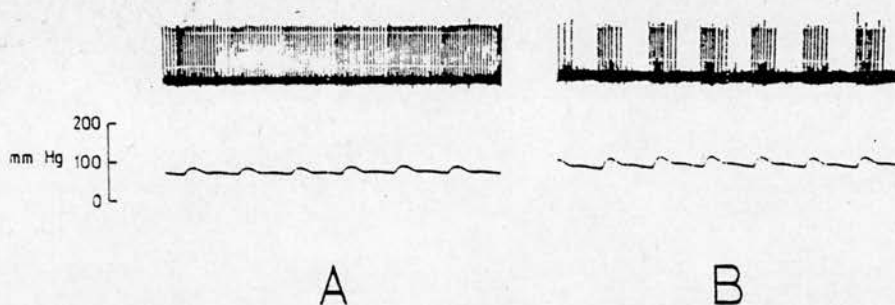


Figure A.1

Two recordings of baroreceptor activity showing fast (A) and slow (B) baroreceptor activity in the rabbit sinus nerve. The two recordings were obtained from different filaments of one sinus nerve. Panels show, from above downwards : action potentials and B.P.

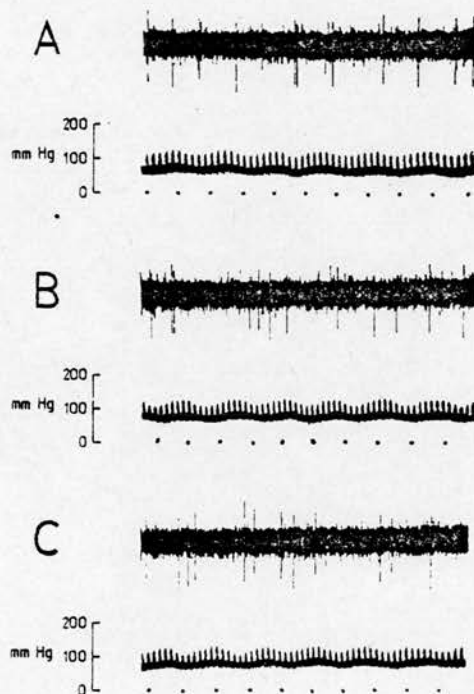


Figure A.2

Recording of unidentified aperiodic activity in the rabbit sinus nerve obtained from an animal breathing room air (A), 5%  $O_2$  in  $N_2$  (B) or 100%  $O_2$  (C). Panels show, from above downwards : action potentials, B.P. and one second time marks.

fibres which conduct afferent chemoreceptor activity in the cat are composed of two-thirds fast-conducting A-fibres (about 3 - 5  $\mu\text{m}$  diameter) and one-third c-fibres (Fidone and Sato, 1969). It is possible that most recordings of chemoreceptor activity in the cat are obtained from the more robust A-fibres while in the rabbit the smaller diameter, slow-conducting fibres are more fragile and are subject to damage during dissection of the nerve.

## 2. Baroreceptor activity

Afferent baroreceptor activity was recognised by its characteristic pattern of discharge, i.e. periodic discharge at a frequency dependent on B.P. and synchronized with pulse pressure. Small filaments of the sinus nerve displaying baroreceptor activity were obtained in almost every experiment but were usually discarded in favour of other filaments in the hope that these would contain chemoreceptor activity.

Baroreceptor activity seemed to be of two types - high frequency or fast activity and low frequency or slow activity (see Figure A.1). Fast activity was characterized by a continuous train of impulses at a frequency which fluctuated in time with pulsatile changes in B.P. (see Figure A.1A) while slow activity was characterized by discrete bursts of activity synchronized with pulse pressure (see Figure A.1B). Both types of baroreceptor activity could be recorded from filaments of the same sinus nerve.

## 3. Non-chemoreceptor aperiodic activity

In most experiments recordings of non-chemoreceptor aperiodic activity were obtained. This type of activity was characterized by a random or near random pattern of discharge. The frequency of

aperiodic activity was not appreciably altered during exposure of the experimental animal to hypoxia or hyperoxia (see Figure A.2) or by I.C. injection of ACh, NaCN, DA or 5-HT. Recordings of normal chemoreceptor and baroreceptor activity could be obtained from nerves in which non-chemoreceptor aperiodic activity was observed. In view of the fact that non-chemoreceptor aperiodic activity was not observed in cats and, as pointed out above, the success rate in obtaining recordings of afferent chemoreceptor activity in rabbits was low it is possible that non-chemoreceptor aperiodic activity arose from chemoreceptors which had ceased to function normally. The source of non-chemoreceptor aperiodic activity in the rabbit sinus nerve was not established in the present study.

#### 4. Phasic activity

On some occasions, a fourth type of activity was observed in small filaments of the rabbit sinus nerve. This activity, which was observed less frequently than the types of activity described in 1-3 above, was phasic activity and was characterised by brief bursts of impulses occurring apparently at random. The source of this phasic activity was not established in the present study.

APPENDIX II

PUBLICATIONS



**The effect of  $\alpha$ -flupenthixol on the response of carotid chemoreceptors to acetylcholine, sodium cyanide and dopamine in the cat**

BY R. J. DOCHERTY and D. S. McQUEEN. *Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ*

Dopamine (DA) may modulate sensory activity of cat carotid chemoreceptors (Zapata, 1975; Osborne & Butler, 1975). If the theory advanced by Osborne and Butler is correct and sensory activity is indeed kept

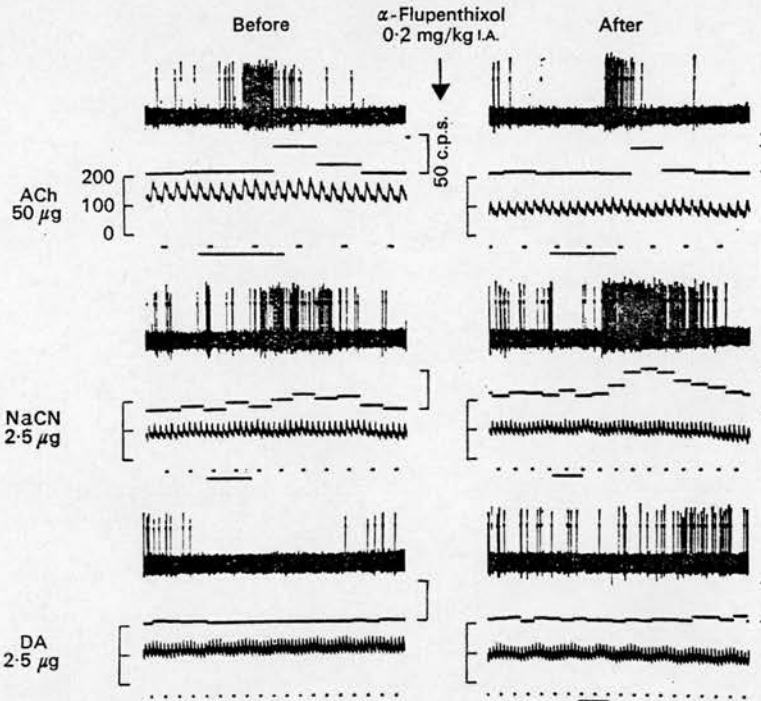


Fig. 1. Response of a chemoreceptor unit before (mean spontaneous discharge  $2.8 \pm 0.1$  c.p.s.) and after ( $2.7 \pm 0.1$  c.p.s.)  $\alpha$ -flupenthixol. Panels show: action potentials; counter output; B.P.; 1 sec and injection markers.

suppressed by the continuous release of DA, then block of the DA receptor should substantially increase spontaneous chemoreceptor activity and also, according to the theory, markedly reduce the response to ACh. We used  $\alpha$ -flupenthixol, a potent inhibitor of DA in the c.n.s. (Iversen, 1975) to block the inhibitory effect of DA.

Experiments were performed on eight pentobarbitone-anaesthetized cats in which ganglio-glomerular nerves were cut, the animals were

[P.T.O.]

artificially ventilated and a gallamine (3 mg/kg) administered. Chemo-receptor activity was recorded from the peripheral end of a sectioned sinus nerve and stimulants injected into the ipsilateral carotid artery. The results showed that  $\alpha$ -flupenthixol (0.2 mg/kg i.a.) abolished the inhibitory effect of DA while the response to NaCN was augmented and that to ACh slightly reduced. (Fig. 1).

Providing that exogenous DA is acting at the same site(s) as endogenous DA, the results suggest that while there may be some tonic inhibition of sensory activity by DA, this is not substantial. It is also unlikely that ACh or NaCN act to any appreciable extent by inhibiting DA release.

This work was supported by an M.R.C. project grant.

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### Inhibitory effects of acetylcholine and dopamine on rabbit carotid chemoreceptors

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During experiments in pentobarbitone-anaesthetized rabbits we observed that intracarotid (i.c.) injection of acetylcholine (ACh, 5-250  $\mu$ g) caused an immediate

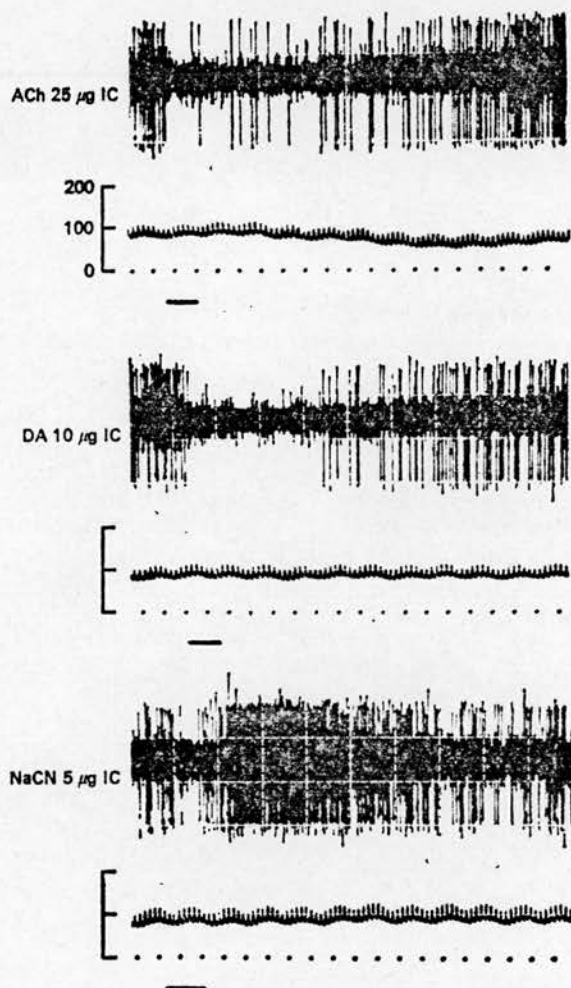


Fig. 1. Recording of chemoreceptor activity obtained from a rabbit, illustrating responses to ACh, DA and NaCN. Panels show: action potentials, B.P.; 1 sec and injection markers.

inhibition of respiration, whereas NaCN (1-25  $\mu$ g i.c.) markedly stimulated respiration. Cutting the ipsilateral sinus nerve abolished the response to NaCN and greatly reduced the inhibitory action of ACh. The possibility that ACh was inhibiting chemosensory activity was investigated by recording from the peripheral end of a cut sinus nerve.

[P.T.O.]

ACh (5–250  $\mu$ g i.c.) caused a dose-dependent inhibition of discharge (Fig. 1). With doses greater than 100  $\mu$ g the inhibition was preceded by a slight transient increase in discharge. Atropine (1–5 mg/kg i.v.) slightly reduced the response to ACh. Dopamine (DA, 10  $\mu$ g i.c.) also inhibited chemoreceptor activity. To determine whether the ACh-induced inhibition was secondary to DA release, we administered the DA antagonist  $\alpha$ -flupenthixol (0.25–0.5 mg/kg i.v.). DA-induced inhibition was abolished, whereas that caused by ACh was only slightly reduced.

In contrast to other species where ACh increases chemosensory activity, and has been proposed as an excitatory transmitter (see review by Biscoe, 1971), our evidence shows that ACh has an inhibitory effect on rabbit chemoreceptors, implying that endogenous ACh is unlikely to be an *excitatory* transmitter in this species.

#### REFERENCE

- BISCOE, T. J. (1971). *Physiol. Rev.* **51**, 437–495.

## INHIBITORY ACTION OF DOPAMINE ON CAT CAROTID CHEMORECEPTORS

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(Received 8 September 1977)

### SUMMARY

1. The influence of some drugs which affect the dopaminergic system was studied on chemosensory responses to dopamine (DA), acetylcholine (ACh), sodium cyanide (NaCN) and hypoxia during experiments on pentobarbitone anaesthetized cats in which chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve.

2. Spontaneous chemosensory activity was inhibited in a dose-dependent manner by DA (0.5–5 µg, i.a.). Higher doses (10–50 µg) caused a delayed increase in discharge and were associated with inconsistent inhibitory responses.

3. The DA antagonist  $\alpha$ -flupenthixol (0.2 mg/kg, i.a.) blocked the inhibitory response to DA without affecting either the spontaneous discharge frequency or the response to ACh. The effect of NaCN was potentiated, and during hypoxia chemoreceptor activity increased more rapidly, although the maximum frequency attained was not appreciably different from control values. Similar results were obtained with haloperidol (0.5 and 1.0 mg/kg, i.v.).

4. Higher doses of  $\alpha$ -flupenthixol (0.5–1.0 mg/kg, i.a.) increased spontaneous chemoreceptor activity, but this was regarded as a non-specific effect of the drug since at these doses the inhibitory effect of 5-hydroxytryptamine (5-HT) was also abolished.

5. The animals were exposed to alternate periods of hypoxia and hyperoxia following administration of the tyrosine hydroxylase inhibitor  $\alpha$ -methyl *p*-tyrosine (AMPT, 0.2–10 mg/kg, i.a.). The inhibitory response previously evoked by amphetamine was abolished, and electron microscopic studies showed a great reduction in the number of dense-cored granules, both of which suggested that DA levels in the carotid body had been substantially reduced. Responses to NaCN and hypoxia were slightly potentiated following AMPT, but neither spontaneous activity nor the response to ACh was affected.

6. Apomorphine (0.05–0.2 mg/kg, i.a.) inhibited the chemoreceptor discharge for up to 45 min, an effect which was antagonized by  $\alpha$ -flupenthixol (0.2 mg/kg, i.a.), implying it resulted from DA receptor stimulation. Although responses to NaCN, hypoxia and higher doses of ACh were reduced following administration of apomorphine, the reduction was not very marked.

7. These results are not compatible with the theory of Osborne & Butler (1975), that in normoxia DA is tonically released in the carotid body and suppresses spontaneous chemosensory activity.



8. It is concluded that DA modulates chemosensory activity by influencing the rate of increase in discharge, without affecting maximum discharge frequency. The mechanism whereby DA is released in response to increased chemosensory activity remains to be established.

#### INTRODUCTION

A new theory of carotid body chemoreceptor activity has recently been advanced (Butler & Osborne, 1975; Osborne & Butler, 1975). This proposes that in normoxic conditions dopamine (DA), which is known to be present in the cat carotid body (Chiocchio, Biscardi & Tramazzani, 1966; Zapata, Hess, Bliss & Eyzaguirre, 1969), is tonically released from Type 1 cells and suppresses the spontaneous discharge of afferent nerve endings; in hypoxic conditions DA release is attenuated and consequently afferent nerve activity increases.

The present pharmacological study was undertaken to test this theory by investigating the response of cat carotid chemoreceptors to acetylcholine (ACh), sodium cyanide (NaCN), DA, and hypoxia, before and after administering drugs which influence the dopaminergic system. A preliminary report on some of the results has been made to the Physiological Society (Docherty & McQueen, 1977).

#### METHODS

The experimental details have previously been described fully (McQueen, 1977) and only a brief summary follows. Experiments were performed on cats of either sex weighing between 2.0 and 4.4 kg (mean weight 2.9 kg) which were anaesthetized with sodium pentobarbitone (42 mg/kg, i.p.), supplemented approximately every 1.5–2 hr during the experiment by 10% of the initial dose administered i.v. Blood pressure was recorded from a femoral artery, the bladder drained at regular intervals and the rectal temperature maintained at  $38 \pm 0.5^\circ\text{C}$ .

A sinus nerve was cut centrally and electrical activity from single or multiple chemoreceptor units was recorded from filaments of the peripheral nerve. The ganglioglomerular nerves were cut in order to eliminate the influence on chemoreceptor discharge of reflex changes in sympathetic nerve activity (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961). The animals were artificially ventilated either with air, 5%  $\text{O}_2$  in 95%  $\text{N}_2$  (hypoxic stimulus), or 100%  $\text{O}_2$  (hyperoxia). They were paralysed by gallamine triethiodide (3 mg/kg i.v.).

Drug solutions (0.1 ml.) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded and washed in with 0.2 ml. modified Locke solution which had been bubbled with 5%  $\text{CO}_2$  in air in a water-bath at  $37^\circ\text{C}$  (solution  $P_{\text{CO}_2}$  32 mmHg;  $P_{\text{O}_2}$  132 mmHg; pH 7.0). Injections were made over a 2 sec period commencing at the peak of the inspiratory phase of the respiratory cycle.

Nerve activity was recorded on magnetic tape (d.c. to 1250 Hz) and subsequently analysed to provide data concerning the change in integrated discharge ( $\Delta \Sigma x$ ) following drug administration. Responses were plotted against  $\log_{10}$  dose and straight lines fitted by the method of least-squares to data obtained before and after the test procedures. A response in the central region of the control dose-response line was selected arbitrarily and the dose of stimulant required to match this response following drug administration was calculated from the post-drug dose-response line. The ratio of the dose required after drug administration to that required in the control state is the *dose ratio*. Dose ratios obtained from different experiments were pooled and data presented as the *mean dose ratio*  $\pm$  s.e. of mean. These ratios provided a quantitative estimate of the effect of a drug on chemoreceptor responses to ACh and NaCN. Control values were determined by calculating the average discharge in the 20 sec period preceding the test stimulus. All the individual values were pooled to provide a mean control  $\pm$  s.e. of the mean for spontaneous chemoreceptor activity which could be compared with that obtained following administration of one of the drugs being studied. Inhibitory responses were calculated by determining the integrated discharge ( $\Sigma x$ ) observed during the response period ( $t$  sec), defined

as the time from onset of inhibition until return to control average discharge ( $\bar{x}$  c.p.s.). The inhibition was then expressed as:

$$-\Delta\Sigma x = \Sigma x - (\bar{x} \cdot t).$$

**Drugs.** Drugs were prepared in modified Locke solution (NaCl 6.0 g; KCl 0.42 g; CaCl<sub>2</sub> 0.24 g; Tris base, 6.0 g; normal HCl 39 ml.; distilled water to 1 l.) excepting  $\alpha$ -flupenthixol and  $\alpha$ -methyl-*p*-tyrosine which were dissolved in 0.9% aqueous sodium chloride, and haloperidol which was dissolved in 1% aqueous tartaric acid.

The drugs used were: sodium pentobarbitone (Abbott Laboratories), gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide (B.D.H.), dopamine hydrochloride (Koch Light),  $\alpha$ -(*cis*)-flupenthixol dihydrochloride (Lundbeck & Co.), apomorphine hydrochloride (Macfarlan Smith), haloperidol (Janssen), 5-hydroxytryptamine creatinine sulphate, D-amphetamine sulphate (Sigma), DL- $\alpha$ -methyl-*p*-tyrosine (Labkemi A.B.).

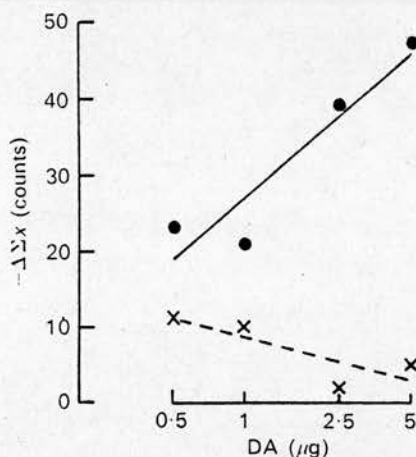


Fig. 1. Dose-response lines showing the inhibitory effect of low doses of DA (plotted on a  $\log_{10}$  scale) on a single chemoreceptor unit before (●—●) and after (x---x) administering  $\alpha$ -flupenthixol (0.2 mg/kg, i.a.). The average spontaneous discharge was  $2.5 \pm 0.3$  c.p.s. before and  $2.8 \pm 0.3$  c.p.s. after  $\alpha$ -flupenthixol. Straight lines were fitted to the data by the method of least-squares.

## RESULTS

Experiments were performed on fifteen cats from which a total of seventeen recordings (eight single, nine multiple units) of chemoreceptor activity were obtained.

### Dopamine

DA reduced spontaneous discharge frequency in all fifteen experiments, the magnitude of the inhibition being dose-dependent over the range 0.5–5  $\mu$ g i.a. (see Fig. 1) and the effect lasting for 5–45 sec. Higher doses (10–50  $\mu$ g i.a.) caused a delayed or secondary increase in discharge which curtailed the primary inhibition and gave inconsistent responses. A secondary increase in chemoreceptor discharge was also observed by Zapata (1975) in his experiments with DA on the carotid body *in vitro*, so it was unlikely that this delayed effect was attributable to vascular changes. Zapata also reported that frequent administration of DA (dose unspecified) caused inhibitory responses to be converted to biphasic responses. In view of his findings and our observations with high doses of DA, we confined our studies to low doses of DA

administered infrequently and obtained consistent responses to both DA and the chemoreceptor stimulants.

Early in experiments ungasged Locke solution caused a very slight inhibition of spontaneous chemoreceptor activity, but later it caused an inhibition similar to that evoked by low doses of DA. It was therefore necessary to use Locke solution which

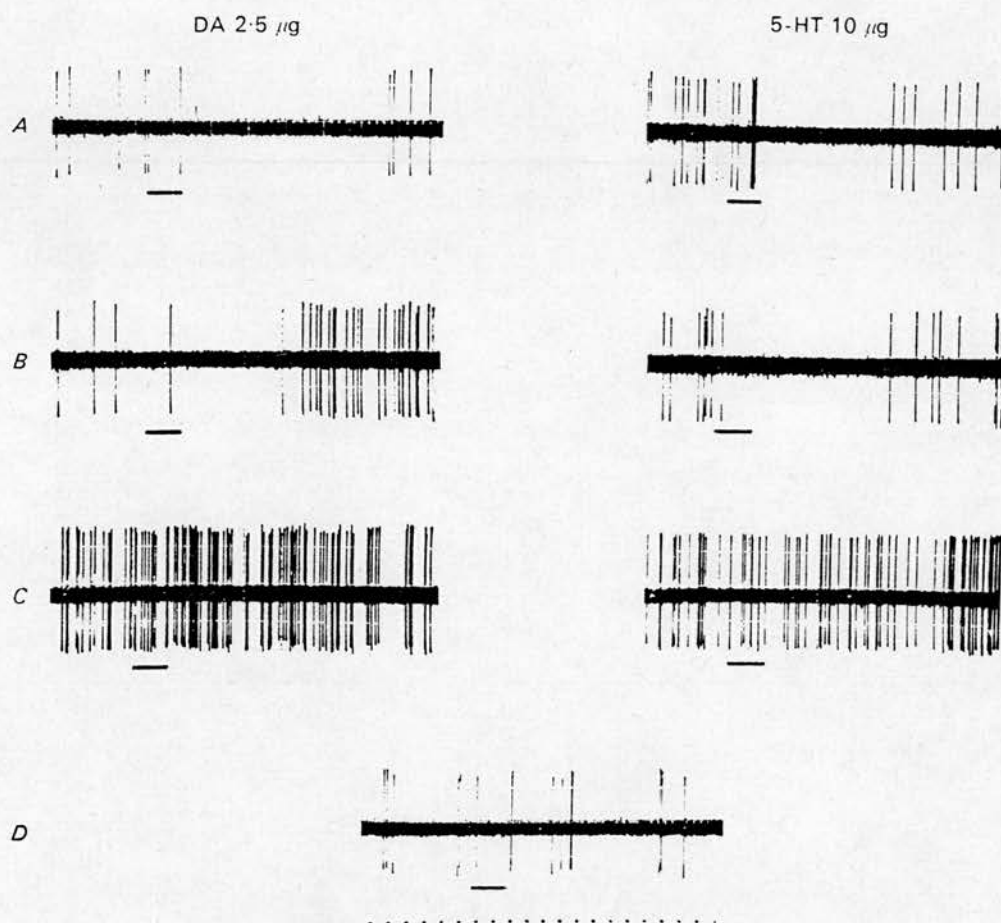


Fig. 2. Responses of a single chemoreceptor unit to DA and to 5-HT before and after  $\alpha$ -flupenthixol. A, control, B, after 0.05 mg/kg  $\alpha$ -flupenthixol, i.a. and C, after an additional 0.5 mg/kg. D shows the effect of 0.3 ml. Locke solution, i.a. Injection periods are represented by the horizontal bar below each panel and 1 sec timing marks are shown below D.

had been bubbled with 5% CO<sub>2</sub> in air to wash in drug solutions (the gassed solution did not cause any appreciable inhibition, even during long experiments; see Fig. 2). There was a slight increase in mean B.P. which commenced about 10 sec after an i.a. injection of high doses of DA (> 10 µg). With lower doses there was no obvious change in mean B.P.

*$\alpha$ -Flupenthixol*

$\alpha$ -Flupenthixol is a potent DA antagonist (Møller Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland & Christensen, 1973; Miller, Horn & Iversen, 1974) which acts post-synaptically (House & Ginsborg, 1976) and has very little anticholinergic activity (Iversen, 1975). We used it to reduce the inhibitory action of DA and, while responses to DA were suppressed, investigated the effects of ACh, NaCN and hypoxia. Dose ratios showing the influence of  $\alpha$ -flupenthixol on the responses were calculated from the central region of  $\Delta\Sigma x$  dose-response lines and the results obtained from ten experiments are summarized in Fig. 3. Responses to DA could not be expressed in the same way because after  $\alpha$ -flupenthixol the slope of the DA dose-response line became negative, reflecting the tendency for DA to evoke excitatory responses under these conditions (see Figs. 1 and 2).

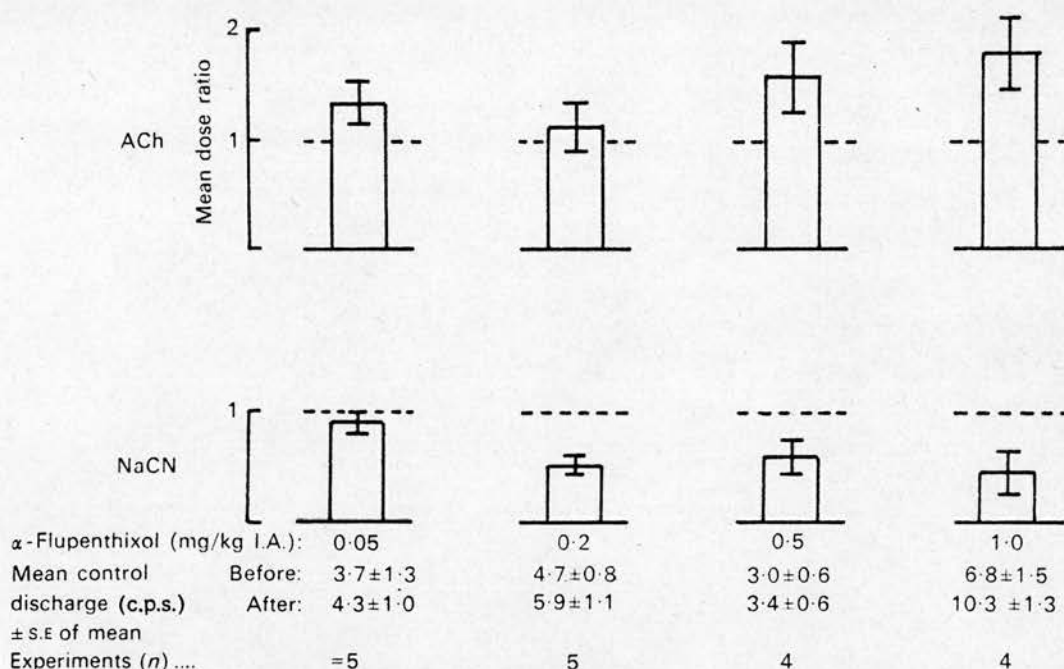


Fig. 3. Pooled dose ratio data showing the effect of various doses of  $\alpha$ -flupenthixol on chemoreceptor responses to ACh and NaCN. Data are presented as mean dose ratios  $\pm$  S.E. of the mean, and the dashed line represents a dose ratio of 1.

Low doses of  $\alpha$ -flupenthixol (0.05 mg/kg, I.A.) reduced the inhibitory action of DA and showed up a small delayed increase in discharge (see Fig. 2), without appreciably affecting either spontaneous chemoreceptor activity or the responses to ACh and NaCN (Figs. 2 and 3). A dose of 0.2 mg/kg, I.A. abolished the inhibitory effect of DA (see Fig. 1), potentiated the response to NaCN, and was without much effect on the spontaneous discharge frequency or the response to ACh. Higher doses of antagonist (0.5 or 1.0 mg/kg, I.A., additional to 0.05 and 0.2 mg/kg respectively) resulted in DA causing a brief period of excitation, and whereas responses to NaCN were still



potentiated, those to ACh were slightly inhibited. There was also a tendency for spontaneous activity to increase, particularly after the 1 mg/kg dose.

Following administration of  $\alpha$ -flupenthixol the rate of increase of chemoreceptor discharge during hypoxia was much greater than in the control, although the maximum frequency reached was not appreciably different in the two states (see Fig. 4B).

It has been reported that besides inhibiting the depressive action of DA in the C.N.S.,  $\alpha$ -flupenthixol also antagonizes the depressive effect of 5-HT, being only slightly more selective against DA (Straughan & Dray, 1976). Accordingly, in the present study responses to both DA and 5-HT were obtained before and after administering  $\alpha$ -flupenthixol so that the specificity of the antagonist on chemosensory responses could be assessed. 5-HT caused a slight increase in discharge on injection followed by a period of inhibition similar to that evoked by DA (see also Nishi, 1975). There was a tendency for low doses of  $\alpha$ -flupenthixol to reduce the excitatory action of 5-HT, although this was not consistent. The inhibitory effect was not affected until doses of 0.5 mg/kg or more were used, when the response was abolished (Fig. 2). This finding meant that results obtained following high doses of  $\alpha$ -flupenthixol ( $\geq 0.5$  mg/kg, i.a.) had to be interpreted cautiously because the drug was evidently no longer acting selectively.

The dopamine antagonist *haloperidol* in cumulative doses of 0.2, 0.5 and 1.0 mg/kg, i.v. was studied in one experiment and the results obtained were similar to those obtained with  $\alpha$ -flupenthixol. The lowest dose was without much effect, but the higher two reduced, but did not abolish, the inhibitory action of DA while potentiating the response to NaCN and inhibiting that to ACh. Spontaneous discharge frequency was  $2.1 \pm 0.2$  c.p.s. before and  $3.1 \pm 0.4$  after 0.5 mg/kg, and  $2.1 \pm 0.3$  c.p.s. after the additional 1 mg/kg dose of haloperidol.

#### *$\alpha$ -Methyl p-tyrosine (AMPT)*

As an alternative to using receptor antagonists to interfere with the actions of endogenous DA, attempts were made to increase the turnover of DA in the presence of AMPT, a drug which blocks the biosynthesis of catecholamines by inhibiting tyrosine hydroxylase. The animals were exposed to two ten min periods of hypoxia separated by a ten min period of hyperoxia over the course of 30 min and they were then returned to breathing room air. This was done before and after administering AMPT in doses of 0.2, 1.0 and 10 mg/kg, i.a. Results from three experiments showed that the low dose slightly potentiated the responses to NaCN and hypoxia, an example of this can be seen in Fig. 4A; higher doses caused a further small increase. Responses to ACh were only very slightly potentiated, even after the highest dose of AMPT. Spontaneous chemoreceptor activity averaged  $1.3 \pm 0.1$  c.p.s. before and  $1.3 \pm 0.2$  c.p.s. after AMPT, 0.2 mg/kg. The corresponding values for 10 mg/kg, i.a. were  $1.3 \pm 0.1$  and  $1.5 \pm 0.1$  c.p.s. Electron microscopy revealed that depletion of dense-cored granules occurred following the 1 mg/kg dose, as compared with untreated carotid bodies exposed to alternating periods of hypoxia and hyperoxia (K. Bell, R. J. Docherty & D. S. McQueen, unpublished observations).



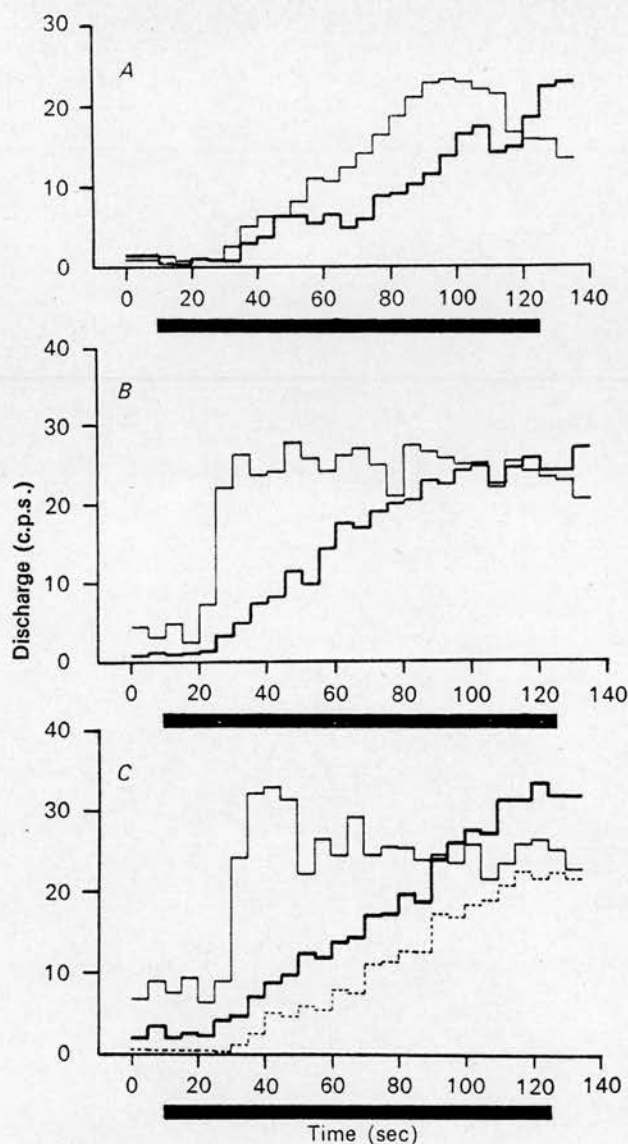


Fig. 4. The upper panel *A* shows the response of a single chemoreceptor unit (discharge averaged over 5 sec intervals) to 120 sec of hypoxia, represented by the horizontal black bar, before (—) and after (---) AMPT 0.2 (mg/kg, i.a.).

The centre panel *B* illustrates the response to hypoxia obtained from a recording containing two chemoreceptor units before (—) and after (---) administering  $\alpha$ -flupenthixol (0.2 mg/kg, i.a.).

In the lower panel *C* the response of another two chemoreceptor units to hypoxia is shown before (—) and after (---) apomorphine (0.2 mg/kg, i.a.). Administration of  $\alpha$ -flupenthixol (0.2 mg/kg, i.a.) reversed the inhibition caused by apomorphine and potentiated the response to hypoxia (---).

*D-Amphetamine*

Amphetamine causes release of DA (Bunney, Aghajanian, & Roth, 1973) and 10  $\mu\text{g}$  was injected i.a. before and after treating the animal with AMPT in two experiments. This dose of amphetamine caused a slight inhibition of chemoreceptor discharge in the control state (although not as marked as that observed following low doses of DA) which was abolished by AMPT 10 mg/kg and hypoxia + hyperoxia.

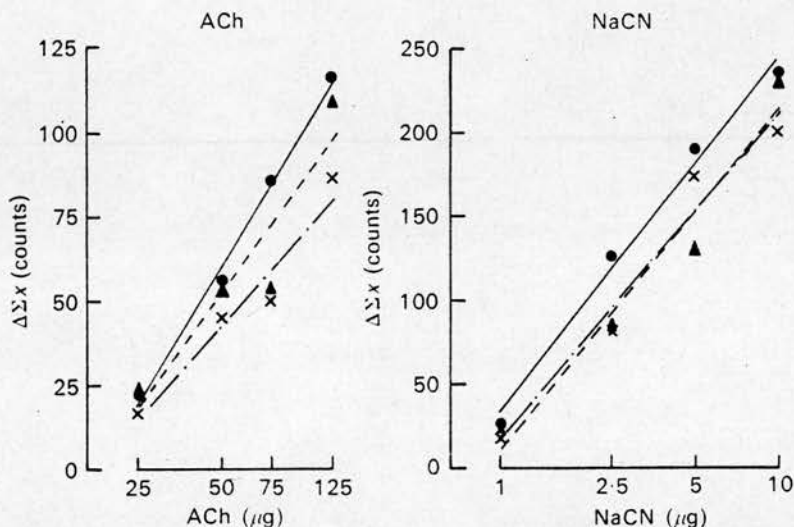


Fig. 5. Dose-response lines, from a recording of chemoreceptor activity (three units), illustrating the influence of apomorphine on responses evoked by ACh and NaCN. Doses are plotted on a  $\log_{10}$  scale and straight lines, fitted to the data by the method of least squares, show: control response ( $\bullet$ — $\bullet$ ), responses after apomorphine, 0.05 mg/kg, i.a. ( $\times$ — $\times$ ), and after a further dose of 0.2 mg/kg, i.a. ( $\blacktriangle$ — $\blacktriangle$ ).

Mean control discharge frequencies were  $3.8 \pm 0.4$  c.p.s. before apomorphine and  $0.9 \pm 0.1$  and  $1.7 \pm 0.2$  after 0.05 and 0.2 mg/kg apomorphine respectively.

*Apomorphine*

Apomorphine was used in two experiments to provide a long-lasting stimulation of the DA receptor. It was studied in cumulative doses of 0.05 and 0.2 mg/kg, i.a. and caused an inhibition of spontaneous chemoreceptor activity which persisted for up to 45 min. This allowed sufficient time to obtain dose-response data for ACh and NaCN. Although background discharge frequency was reduced, DA could still evoke an inhibitory response. Similar results were obtained in both experiments, dose-response lines from one of them being shown in Fig. 5 where it can be seen that responses to NaCN were slightly inhibited as were responses to ACh, particularly at the higher doses. The rate of increase of discharge during hypoxia was reduced following apomorphine, an effect which was reversed by  $\alpha$ -flupenthixol (see Fig. 4C).

## DISCUSSION

The present results confirm that DA inhibits chemosensory activity following close-arterial injection to the cat carotid body (see also Black, Comroe & Jacobs, 1972; Sampson, 1972; Zapata, 1975). This inhibitory effect of DA is primarily a direct

action on the chemoreceptors and not a consequence of vascular changes (Zapata, 1975; Sampson, Aminoff, Jaffe & Vidruk, 1976a; Sampson & Vidruk, 1977). Higher doses of DA caused a delayed increase in discharge which greatly reduced the duration of the inhibitory response. This increase seems similar to that observed *in vitro* by Zapata (1975) which was unaffected by dopaminergic or  $\alpha$ -adrenergic blockers. The cause of the delayed increase was not investigated in the present study and remains to be determined.

Recently Sampson & Vidruk (1977) obtained reproducible DC potential changes with DA, even when administered every 3–4 min (dose unspecified), using the *in vitro* carotid body preparation, and they contrasted their results with Zapata's finding that frequent administration of DA reduced the chemoreceptor inhibitory response. However, whether results obtained using the mass receptor potential can be compared meaningfully with those using chemosensory discharge frequency has yet to be established. Sampson (1972) and Sampson *et al.* (1976a) apparently obtained repeatable inhibitory responses to DA *in vivo* using doses of 2–5  $\mu$ g, which accords with our results at these doses, but they did not study the higher doses which we found gave delayed increases in discharge and inconsistent inhibitory effects.

If Osborne & Butler's theory is correct (see Introduction), chemoreceptor activity should increase when the inhibitory action of DA is blocked because spontaneous depolarization of sensory nerve endings would no longer be suppressed. Furthermore, according to their theory ACh is released from sensory nerve endings in the carotid body and acts to suppress the release of DA from Type 1 cells, thereby causing an increase in chemoreceptor discharge. In a situation where the inhibitory effect of DA has been blocked, ACh should no longer cause excitation. Our results show that blocking the inhibitory effect of DA with  $\alpha$ -flupenthixol (0.2 mg/kg, i.a.) did not affect either spontaneous chemoreceptor activity or the response to ACh. Higher doses (e.g.  $\geq 0.5$  mg/kg, i.a.) did cause spontaneous activity to increase and reduced the responses to ACh, but at this dose inhibitory responses to 5-HT were also blocked, implying that the drug was not acting selectively against DA, which we assumed it was at lower doses. It should be noted that  $\alpha$ -flupenthixol has weak  $\alpha$ -adrenergic blocking actions (Møller Nielsen *et al.* 1973). After  $\alpha$ -flupenthixol, 0.2 mg/kg, responses to NaCN were potentiated and the increase in discharge during hypoxia was much more rapid. Similar results were obtained with another DA antagonist, haloperidol.

In addition to the experiments with DA antagonists, we also studied the response of the chemoreceptors during prolonged DA receptor stimulation. Inhibitory responses to DA injections were short-lasting, and although the use of DA infusion to give a sustained inhibition was considered, this was rejected because of the possibility of causing secondary increases in discharge. Instead the DA agonist apomorphine (Bunney *et al.* 1973) was used and gave a long-lasting inhibition of spontaneous chemosensory activity. Responses to NaCN and hypoxia were reduced by apomorphine, as were those to ACh, but to a lesser extent. These results showed that the responsiveness of the chemoreceptors to stimulants was only slightly reduced by prolonged DA receptor stimulation. The inhibitory effect of apomorphine was prevented by  $\alpha$ -flupenthixol, an observation which provides indirect evidence that apomorphine and DA were acting at the same site(s).



We also tried a different approach to testing the theory, the objective being to reduce endogenous DA levels in the carotid body using AMPT. Hypoxia reduces the DA content of the carotid body, both when the sinus nerve is intact (Sampson, Nicolaysen & Jaffe, 1975) and when denervated (Hellström, Hanbauer & Costa, 1976), and the presence of the tyrosine hydroxylase inhibitor AMPT (Moore & Dominic, 1971) should reduce DA biosynthesis during and after hypoxia. In our experiments AMPT was administered and the animals subjected to alternate periods of hypoxia and hyperoxia (the latter because according to Osborne & Butler's theory DA turnover should be greatest when the oxygen tension is higher than normal). The results indicated that AMPT treatment slightly potentiated the response to NaCN but had little effect on either spontaneous activity or the response to ACh. To test for DA depletion we studied the inhibitory effect of D-amphetamine, which was presumed to reflect DA release (Bunney *et al.* 1973) and which was reduced or abolished by AMPT. We also assessed, qualitatively, the reduction by AMPT in the dense-cored vesicle content of Type 1 cells, where DA is thought to be stored (Chen & Yates, 1969; Kobayashi, 1971; see also the review by Biscoe, 1971). Although Zapata *et al.* (1969) reported that prolonged hypoxia in cats had no effect on either the dense-cored vesicles or the catecholamine levels in the carotid body, more recent studies by Hellström *et al.* (1976) have shown that 15 min of hypoxia (5% O<sub>2</sub> in N<sub>2</sub>) selectively reduced the DA content in the rat carotid body.

We have interpreted the results as showing that DA levels in the carotid body were greatly reduced by the catecholamine-synthesis inhibitor AMPT, although other interpretations are possible. For example, structural changes in the Type 1 cells may reflect changes in the amount of complexing substances rather than DA content, and noradrenaline in the cat carotid body (Chiocchio *et al.* 1966) may also be affected by hypoxia (Mills & Slotkin, 1975; cf. Zapata *et al.* 1969), AMPT and amphetamine. However, responses to stimulants following the combination of AMPT and hypoxia/hyperoxia were in good agreement with results obtained using the DA antagonist  $\alpha$ -flupenthixol, which adds further support to the interpretation that AMPT was reducing DA levels.

In summary, the present results demonstrate that responsiveness of the chemoreceptors to stimulants was only slightly affected when the dopaminergic system was influenced by blocking drugs ( $\alpha$ -flupenthixol or haloperidol), a biosynthesis inhibitor (AMPT), or a long-acting DA agonist (apomorphine). These findings do not support the theory of Osborne & Butler (1975) that DA is tonically released in the carotid body to suppress chemosensory activity. There is also evidence in the literature which is at variance with this theory. For example, reserpine treatment does not modify the response to chemoreceptor stimulants, even though DA levels in the carotid body are substantially reduced (Eyzaguirre & Zapata, 1968; Zapata *et al.* 1969; Nishi, 1975). Also, Zapata (1975) found that the DA antagonist spiroperidol did not increase spontaneous chemosensory activity *in vitro* and he concluded that 'a continuous release of DA to maintain tonic inhibition of chemosensory fibres is not very probable'.

Our results are, however, compatible with suggestions that DA may, through its inhibitory action, *modulate* chemosensory activity (Mitchell & McDonald, 1975; Zapata, 1975). We consider that increased afferent activity causes a release of DA

which reduces the sensitivity of the sensory nerves; this does not preclude the possibility that stimulants (e.g. hypoxia) also cause a direct release of DA from storage sites. In this scheme DA release would be minimal in the normal resting state, which explains why  $\alpha$ -flupenthixol and AMPT had no appreciable effect on spontaneous discharge frequency. Responses to ACh injections last for only 1–2 sec (not very physiological) which may not be long enough to allow any DA released by the increased activity to exert an inhibitory influence, thus explaining why responses to ACh were not greatly affected by the DA inhibitors. Responses to NaCN and to hypoxia are longer-lasting (4–10 sec for NaCN) and were evidently suppressed by DA, since they were potentiated following  $\alpha$ -flupenthixol or AMPT. We conclude, therefore, that DA modulates an increase in chemosensory activity by influencing the rate of increase in discharge, but not the maximum discharge frequency. The mechanism whereby DA is released in response to increased chemosensory activity remains to be established, but may involve reciprocal synapses, possibly utilizing ACh (Hess & Zapata, 1972; McDonald & Mitchell, 1975), or release from Type 1 cells by increased efferent activity in the sinus nerve (Biscoe & Stehbens, 1967; Biscoe, Lall & Sampson, 1970; Neil & O'Regan, 1971; Sampson, 1972; Sampson, Aminoff, Jaffe & Vidruk, 1976b), although the physiological importance of the latter pathway has been questioned (McCloskey, 1975; Mitchell & McDonald, 1975).

This work was supported by a project grant from the Medical Research Council. R. J. Docherty is in receipt of an M.R.C. Research Studentship. We are grateful to Lundbeck & Co. for generously supplying  $\alpha$ -flupenthixol.

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## THE EFFECTS OF ACETYLCHOLINE AND DOPAMINE ON CAROTID CHEMOSENSORY ACTIVITY IN THE RABBIT

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(Received 3 August 1978)

### SUMMARY

1. Intracarotid (I.C.) injection of either acetylcholine (ACh) or dopamine inhibited spontaneous chemosensory activity recorded from the peripheral cut end of the sinus nerve in the anaesthetized rabbit.

2. High doses of ACh ( $\geq 50 \mu\text{g}$  I.C.) evoked a slight increase in discharge which preceded the inhibition. This excitation was attributable to a nicotinic action of the drug since it was abolished by mecamylamine.

3. The muscarinic agonist bethanechol inhibited chemoreceptor activity, an effect which was blocked by high doses of atropine, as was the inhibition caused by ACh. Dopamine-induced inhibition was unaffected by atropine.

4. Atropine, in doses sufficient to abolish the vasodepressor effect of ACh, only slightly reduced the inhibitory action of ACh on the chemoreceptors. Also, the vasodilators sodium nitrite and sodium nitroprusside did not appreciably alter chemosensory discharge. It seems unlikely, therefore, that the inhibitory response to ACh is secondary to vascular changes.

5. The inhibitory response to dopamine, but not that to ACh, was blocked by the dopamine antagonist  $\alpha$ -flupenthixol. This implies that inhibition of chemosensory activity evoked by exogenous ACh was not secondary to dopamine release.

6. The implications of the results are discussed, particularly with regard to the possible physiological role of ACh as a modulator of carotid chemosensory activity.

### INTRODUCTION

The present electrophysiological investigation was undertaken to examine the effects of acetylcholine (ACh) and dopamine on carotid chemosensory activity in the rabbit. It is well known that ACh stimulates arterial chemoreceptors in the cat (e.g. see Anichkov & Belen'kii, 1963; Schweitzer & Wright, 1938) and dog (e.g. Heymans, Bouckaert, Farber & Hsu, 1936), but there do not appear to be any reports concerning the effects of ACh, or other drugs which affect the cholinergic system, on the activity of rabbit carotid body chemoreceptors.

Dopamine seems to have species-dependent effects on the carotid chemoreceptors, stimulating sensory activity in dogs (Jacobs & Comroe, 1968), but inhibiting it in cats (Black, Comroe & Jacobs, 1972; Sampson, 1972; Zapata, 1975; Docherty & McQueen, 1978a). We were interested in determining what effect dopamine has on chemosen-

sory activity in the rabbit, particularly since it is present in the carotid body of this species (Dearnaley, Fillenz & Woods, 1968). A preliminary account of some of this work has been presented to the Physiological Society (Docherty & McQueen, 1978b).

#### METHODS

Experiments were performed on male rabbits (New Zealand White or Californian) weighing between 2.5 and 3.5 kg (mean weight = 2.9 kg).

*Anaesthesia.* Animals were anaesthetized with sodium pentobarbitone (30–50 mg/kg) or urethane (400 mg/kg) and  $\alpha$ -chloralose (6 ml./kg of a 1% solution in saline), administered through an ear vein, with supplements as required (Korner, Uther & White, 1968).

*General.* A cannula was inserted in the trachea low in the neck. Blood pressure was measured via a pressure transducer (Bell & Howell, 4-422) from a cannulated femoral artery, displayed on a pen recorder (Devices, M4), and recorded by an FM tape recorder (Tandberg, 100; frequency response d.c. to 1250 Hz). Arterial blood pH,  $P_{O_2}$ , and  $P_{CO_2}$  were measured at hourly intervals using a Radiometer gas monitor (BMS 3 with PHM 71 meter). A femoral vein was cannulated and used for drug administration. Rectal temperature was monitored and maintained at  $39 \pm 0.5^\circ\text{C}$  by a heating pad.

The carotid bifurcation region was exposed and dissected free from surrounding tissue. A cannula was inserted into the lingual artery and advanced until its tip lay in the common carotid artery approximately 1.5 cm caudal to the carotid bifurcation. This cannula was used for close-arterial administration of drugs to the carotid body.

*Recording sinus nerve activity.* The animal was artificially ventilated with room air by a respiratory pump (S.R.I.) operating at 38 strokes/min and gallamine triethiodide (3 mg/kg i.v.) was administered to paralyse spontaneous respiration. This dose had no appreciable effect on chemoreceptor responses to ACh or dopamine. In most experiments end-tidal  $CO_2$  was continuously monitored by an infra-red  $CO_2$  analyser (Med 1A; Grubb Parsons) and the stroke volume of the pump adjusted to maintain end-tidal  $CO_2$  at 5%. The carotid sinus nerve ipsilateral to the catheterized lingual artery was cut centrally and activity in the nerve was recorded as described previously (McQueen, 1977). Chemoreceptor units were identified by their random pattern of discharge, their increase in discharge frequency following injection of sodium cyanide (5  $\mu\text{g}$ ) into the ipsilateral common carotid artery, their increase in discharge in response to hypoxia (breathing 5% oxygen in nitrogen), and by the inhibition of discharge in response to hyperoxia (breathing 100% oxygen).

*Drug administration.* Intracarotid (i.c.) injections of drugs were made in a volume of 0.1 ml. and the catheter (dead space = 0.1 ml.) flushed with 0.2 ml. modified Locke solution at  $37^\circ\text{C}$  which had been bubbled with a 5% carbon dioxide/95% air gas mixture. Injections were made at the peak of the inspiratory phase of the respiratory cycle and completed over one respiratory cycle. i.v. injections of drugs were made in a volume of 0.2–1.0 ml. and the catheter flushed with 0.5 ml. saline. In experiments with mecamlamine, the appropriate volume of dextran solution (2.5% dextran, 5% glucose in distilled water) required to maintain blood pressure at the control level, was administered i.v. This treatment prevented the sustained fall in blood pressure which would otherwise have accompanied administration of this ganglion blocking drug.

*Data analysis.* Action potentials were counted and data analysed as previously described (McQueen, 1977). Responses to drugs were expressed as the absolute difference in discharge following drug administration where

$$\Delta\Sigma x = \Sigma x (\text{response}) - \Sigma x (\text{control})$$

and

$$\Sigma x (\text{control}) = \bar{x} (\text{control}) \times t,$$

$$\Delta\Sigma x = \text{absolute difference in discharge,}$$

$$\Sigma x = \text{total discharge,}$$

$$\bar{x} = \text{average discharge (counts per second),}$$

$$t = \text{response duration (sec).}$$

*Drugs.* Drugs were prepared in modified Locke solution (NaCl 6.0 g; KCl 0.42 g;  $CaCl_2$  0.24 g; Tris base 6.0 g; N-HCl 39 ml.; distilled water to 1 l.; pH = 7.41 at  $37^\circ\text{C}$ ), excepting  $\alpha$ -flupen-

thioxol which was prepared in 0.9% (w/v) sodium chloride solution. Doses referred to are those of the salts.

The drugs used in this investigation were: pentobarbitone sodium, gallamine triethiodide (May & Baker); acetylcholine iodide, sodium cyanide, atropine sulphate; urethane (ethyl carbamate),  $\alpha$ -chloralose, sodium nitrite (B.D.H.); bethanechol chloride, dopamine hydrochloride (Koch-Light); sodium nitroprusside (Griffin & Tatlock); mecamlamine hydrochloride (M.S.D.); 5-hydroxytryptamine creatinine sulphate (Labkemi A. B.); suberyldicholine di-iodide (kindly supplied by Dr A. Ungar, Department of Pharmacology, University of Edinburgh).

## RESULTS

Experiments were performed on twenty-one rabbits from which twenty-three recordings (three single and twenty multiple units) of chemoreceptor activity were obtained.

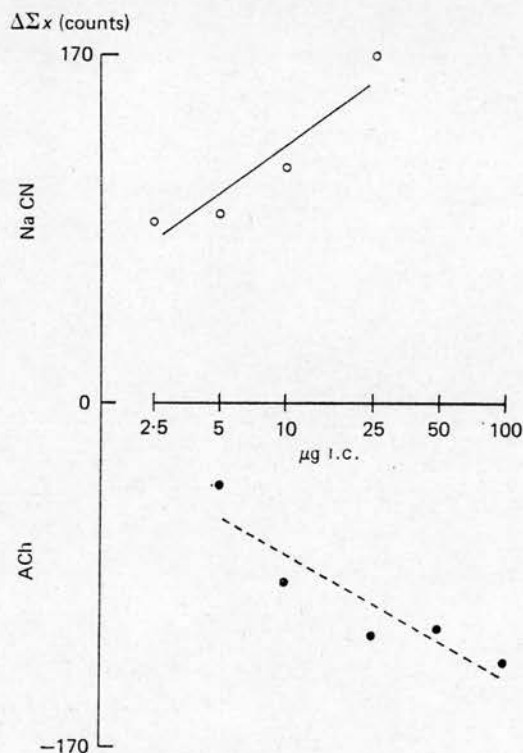


Fig. 1. Dose-response data for sodium cyanide, NaCN ( $\bigcirc$ — $\bigcirc$ ) and acetylcholine, ACh ( $\bullet$ — $\bullet$ ) in one experiment (three chemoreceptor units). In this and subsequent Figures doses ( $\mu\text{g i.c.}$ ) are plotted on a  $\log_{10}$  scale and responses expressed as  $\Delta\Sigma x$  unless otherwise indicated (see Methods section). Lines were fitted to the data by the method of least squares.

### Responses to cholinergic agonists

*Acetylcholine.* Injections of ACh (1–250  $\mu\text{g i.c.}$ ) caused an immediate, short-lasting (1–30 sec) inhibition of chemoreceptor activity in all the experiments, including two in which the ipsilateral superior cervical ganglion had been removed. There was an approximately linear relationship between  $\log_{10}$  dose and response ( $\Delta\Sigma x$ ) over the range 5–100  $\mu\text{g i.c.}$  (see Fig. 1). Doses in this range, which always caused inhibition



of chemoreceptor activity in the rabbit (either anaesthetic), are comparable to doses which stimulate chemoreceptor activity in the cat (McQueen, 1977). With high doses of ACh ( $\geq 50 \mu\text{g}$  i.c.), the inhibition was preceded by a slight (2–3 times control discharge), transient period of stimulation lasting less than 1 sec (see Fig. 2).

*Mecamylamine.* Mecamylamine is a potent ganglion blocking drug (Stone, Torchiana, Navarro & Beyer, 1956; Bennet, Tyler & Zaimis, 1957), which can block the increase in chemoreceptor activity evoked by ACh in the cat carotid body, both *in vitro* (Eyzaguirre & Zapata, 1968) and *in vivo* (Sampson, 1971; McQueen, 1977).

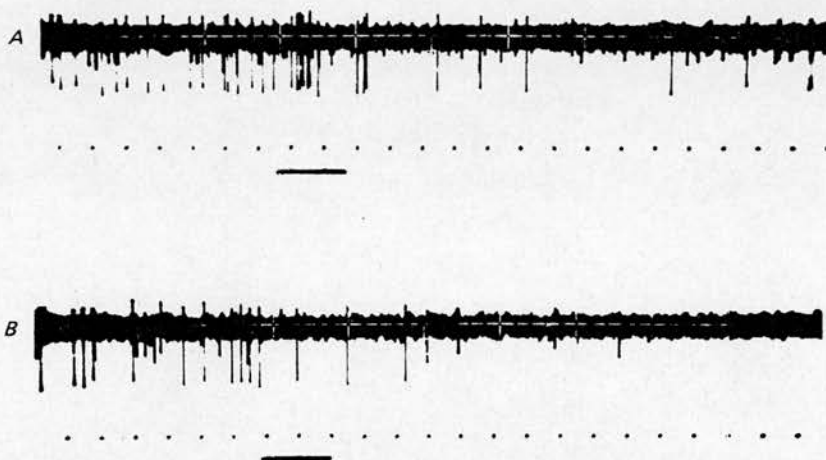


Fig. 2. Chemoreceptor units from an experiment showing the early part of the response to a high dose of ACh ( $50 \mu\text{g}$  i.c.), before (A) and after (B) administration of mecamylamine ( $0.5 \text{ mg/kg}$  i.c.). Panels show from above downwards: action potentials, 1 sec time markers, injection marker.

The inhibition of chemoreceptor activity evoked by ACh in rabbits was either unaffected or potentiated following the administration of mecamylamine ( $1\text{--}5 \text{ mg/kg}$  i.v. or  $0.25\text{--}0.5 \text{ mg/kg}$  i.c.). The transient stimulation seen with high doses of ACh was, however, abolished (Fig. 2).

*Suberyldicholine (SDCh).* SDCh is a potent nicotinic agonist which has been shown to stimulate the carotid chemoreceptors of the cat (Anichkov & Belen'kii, 1963; Dardymov & Ger, 1964; McQueen, 1974). Intracarotid injection of SDCh ( $10\text{--}50 \mu\text{g}$ ) in the rabbit had no consistent effect on carotid chemoreceptor activity.

*Atropine.* Atropine, in doses ( $1\text{--}10 \text{ mg/kg}$  i.v.) sufficient to block the depressor effects of ACh, caused only a slight reduction in the inhibition of chemoreceptor activity produced by ACh (see Fig. 3). There was a large variation in the dose of atropine required to block the vascular effects of ACh in different animals. However, administration of atropine close-arterial to the carotid body ( $1 \text{ mg/kg}$  i.c.) caused a substantial reduction in the chemoreceptor response to ACh (Fig. 4A).

*Bethanechol.* Bethanechol is a muscarinic agonist which is relatively free of nicotinic actions (Molitor, 1936). i.c. injection of bethanechol ( $10\text{--}100 \mu\text{g}$ ) produced a dose-related inhibition of chemoreceptor activity which was greatly reduced by administration of atropine ( $1 \text{ mg/kg}$  i.c.; see Fig. 4B). Unlike the response to ACh, the inhibition was not preceded by a stimulant effect at high doses.



*Responses to sodium cyanide*

Sodium cyanide increased carotid chemosensory activity in all the experiments, including the two in which the ipsilateral superior cervical ganglion had been extirpated. The threshold dose of stimulation was about  $2.5 \mu\text{g}$  i.c. and a maximum response was elicited by  $25 \mu\text{g}$  i.c. The effective dose range is comparable to that for stimulation of carotid chemoreceptors in the cat (McQueen, 1977). Fig. 1 shows

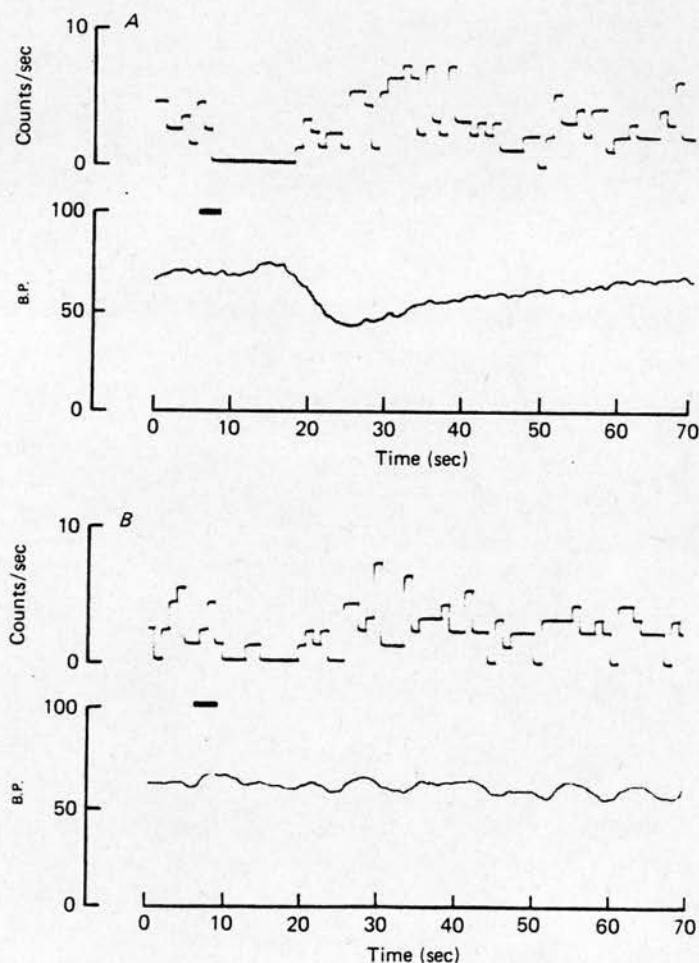


Fig. 3. Response of carotid chemoreceptors (three units) to ACh ( $10 \mu\text{g}$  i.c.) before (A) and after (B) administration of atropine ( $10 \text{ mg/kg}$  i.v.). Panels show from above downwards: discharge frequency (counts/sec), injection marker, mean blood pressure (mmHg), time (sec).

dose-response data for sodium cyanide, illustrating the approximately linear relationship between  $\log_{10}$  dose and the response ( $\Delta\Sigma x$ ) over this range.

The chemoreceptor response to sodium cyanide was not appreciably affected by administration of either atropine or mecamylamine, although in some experiments

there was a slight reduction in the response to sodium cyanide in the presence of mecamlamine (see Figs. 5A, B).

#### Response to dopamine and 5-hydroxytryptamine

**Dopamine.** The effect of dopamine on carotid chemoreceptor activity was studied in sixteen experiments. A single injection (5–10  $\mu\text{g}$  i.c.) caused an immediate, short-lasting (5–20 sec) inhibition of chemoreceptor activity in every experiment (see Fig. 7). The inhibition was sometimes followed by excitation, a phenomenon which

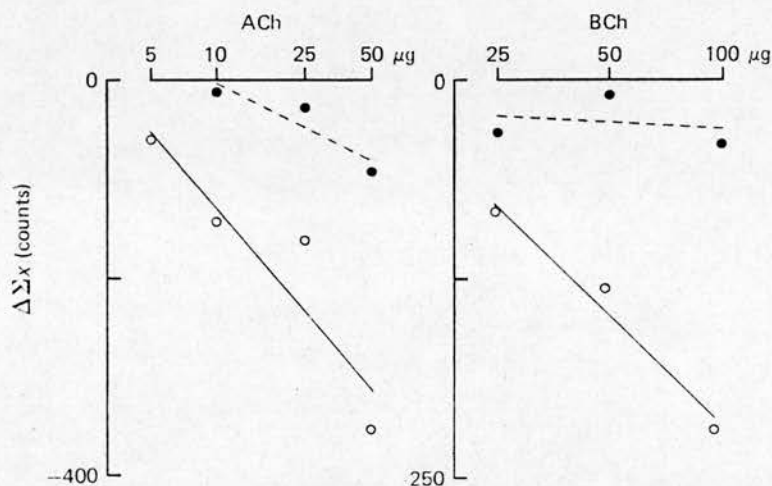


Fig. 4. Dose-response data from one experiment (three units) for ACh and bethanechol (BCh), before (○—○) and after (●—●) atropine (1 mg/kg i.c.).

has also been observed in cats (Zapata, 1975, 1977; Docherty & McQueen, 1978a). The chemoreceptor response to dopamine was unaltered by mecamlamine (0.5–5 mg/kg i.v. or 0.25–0.5 mg/kg i.c.) or atropine (1–5 mg/kg i.v. or 1 mg/kg i.c.).

**$\alpha$ -Flupenthixol.**  $\alpha$ -Flupenthixol is a potent dopamine antagonist (Møller Nielsen, Pederson, Nymark, Franck, Boeck, Fjalland & Christensen, 1973) which has been shown to block the inhibitory action of dopamine on carotid chemosensory discharge in the cat (Docherty & McQueen, 1978a). Administration of  $\alpha$ -flupenthixol (0.25–1 mg/kg i.v. or 0.25–0.5 mg/kg i.c.) in rabbits blocked the inhibitory action of dopamine on carotid chemosensory activity (see Fig. 6A) but did not reduce the inhibitory response to ACh (see Fig. 6B). The excitatory response to sodium cyanide was potentiated by  $\alpha$ -flupenthixol (Fig. 5C).

**5-Hydroxytryptamine (5-HT).** The effect of 5-HT on carotid chemosensory activity was studied in eight experiments. Intracarotid injection of 5-HT (5–10  $\mu\text{g}$ ) caused an intense but transient stimulation of chemoreceptor activity followed by a period of relative inhibition (5–15 sec; see Fig. 7). Both the excitatory and inhibitory components of the response were subject to considerable variation in any experiment, making an accurate assessment of the effects of blocking drugs difficult. The response did not appear to be modified by either mecamlamine or atropine. Low doses of  $\alpha$ -flupenthixol (0.25–0.5 mg/kg i.v.) had little effect on the chemoreceptor response to 5-HT although responses to dopamine were blocked. Higher doses of  $\alpha$ -flupenthixol

(0.25–0.5 mg/kg i.c.) blocked the inhibitory component of the response to 5-HT, this being similar to the block of 5-HT inhibition observed in cats following higher doses of  $\alpha$ -flupenthixol (Docherty & McQueen, 1978a).

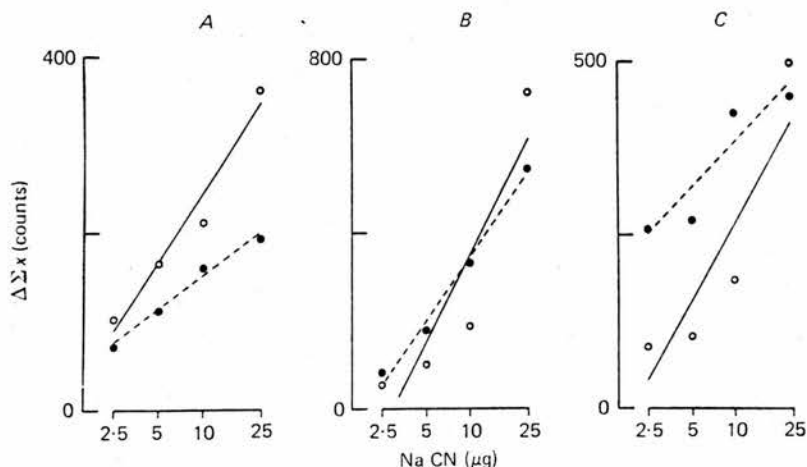


Fig. 5. Dose-response data for NaCN, obtained during three experiments, before (○—○) and after (●—●), *A*, mecamylamine (0.25 mg/kg i.c.), *B*, atropine (2 mg/kg i.v.) and, *C*,  $\alpha$ -flupenthixol (0.25 mg/kg i.v.). Data were from recordings of three, four and three chemoreceptor units respectively.

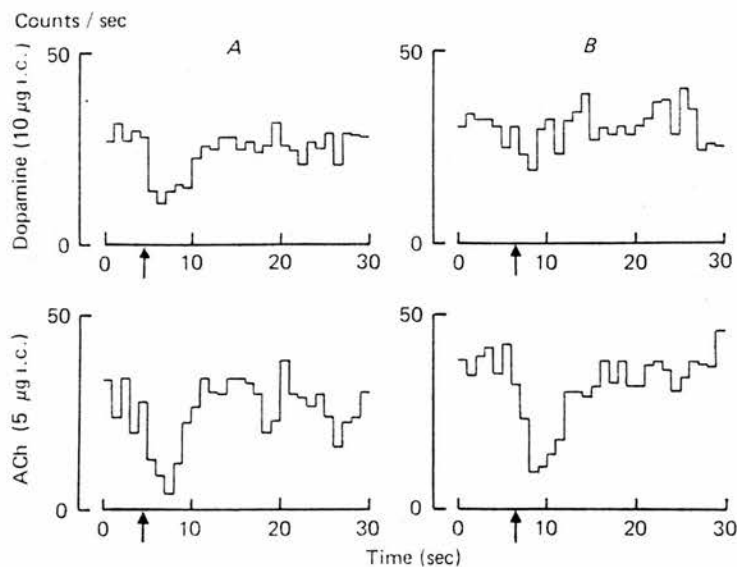


Fig. 6. Effects of dopamine (10  $\mu$ g i.c.) and ACh (5  $\mu$ g i.c.) on spontaneous chemoreceptor discharge (counts/sec), before (*A*) and after (*B*) administration of  $\alpha$ -flupenthixol (0.5 mg/kg i.v.). Injections of ACh or dopamine were made at the arrows. Panels show from above downwards: discharge frequency (counts/sec), time (sec).

*Response to vasodilator substances*

*Sodium nitrite.* Sodium nitrite is a potent vasodilator which acts directly on vascular smooth muscle (Weiss, Wilkins & Haynes, 1937). Injection of sodium nitrite (25–100  $\mu\text{g}$  i.c.) evoked a very slight inhibition of chemoreceptor activity lasting 3–4 sec, but the magnitude of the inhibition did not appear to be dose-related and was no greater than that caused by i.c. injection of an equal volume of modified Locke solution (0.1 ml.).

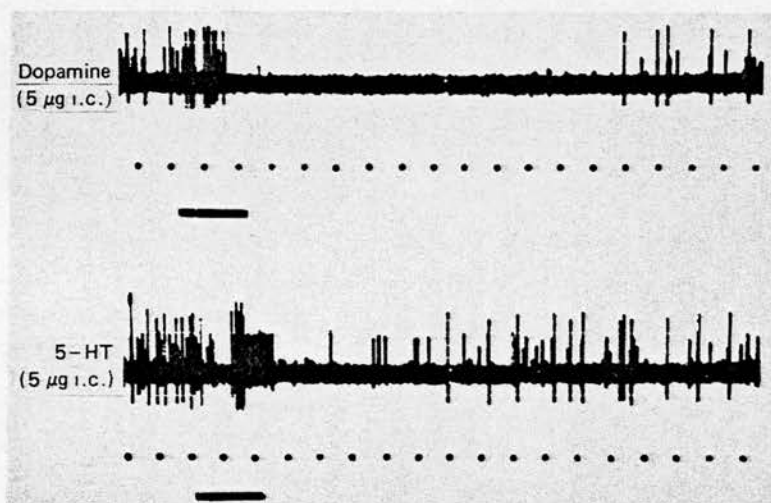


Fig. 7. Chemoreceptor units from an experiment showing the response to dopamine (5  $\mu\text{g}$  i.c.) and 5-hydroxytryptamine (5-HT) (5  $\mu\text{g}$  i.c.). Details as for Fig. 2.

*Sodium nitroprusside.* Like sodium nitrite, sodium nitroprusside is a vasodilator which acts directly on vascular smooth muscle (Johnson, 1929). Sodium nitroprusside (10–50  $\mu\text{g}$  i.c.) evoked a feeble, short lasting inhibition of chemoreceptor activity, independent of dose, followed by a secondary stimulation which was dose-dependent. The magnitude of the initial inhibition evoked by sodium nitroprusside was no greater than that caused by a control injection of an equal volume (0.1 ml.) of modified Locke solution.

## DISCUSSION

The results show that ACh and dopamine both inhibit spontaneous carotid chemosensory activity in anaesthetized rabbits. The inhibitory response to ACh was unexpected, being in complete contrast to the stimulant action obtained in other species (see Introduction). Experiments were performed to investigate the mechanisms whereby the drugs cause inhibition of chemoreceptor activity. The results obtained are discussed below.

*Vascular effects.* Inhibition of chemoreceptor discharge could have resulted from an action of ACh on vascular smooth muscle, although the short latency to onset of the effect argues against this, at least for the early part of the response. Vasodilatation of the carotid body vasculature would increase blood flow into the glomus and might thereby be expected to reduce spontaneous chemosensory discharge frequency before



any tendency for discharge to increase secondary to the delayed fall in B.P. caused by circulation of the ACh (see Fig. 3), although autoregulation of blood flow that can occur in the rabbit carotid body (McCloskey, 1968) complicates the speculation. The results from our experiments show that whereas a low dose of atropine blocked the ACh-induced fall in systemic B.P., the inhibitory response of the chemoreceptors to ACh was only slightly reduced. The vascular explanation therefore seemed unlikely, providing that the carotid body vascular responsiveness to ACh is similar to that of the peripheral vasculature. This may or may not be the case. However, the potent vasodilators sodium nitrite and sodium nitroprusside also had little effect on chemosensory activity when administered close-arterial to the carotid body, the increase in discharge associated with nitroprusside probably being due to the production of cyanide ions during the conversion of nitroprusside to thiocyanate. These results with atropine and the vasodilator drugs indicate that although vasodilatation may explain a small part of the inhibition of chemoreceptor activity evoked by ACh, it is not responsible for the greater part of the effect. This view is supported by the recent finding that ACh inhibits chemosensory activity of the superfused rabbit carotid body *in vitro* (Monti-Bloch & Eyzaguirre, 1977), this being a preparation in which vascular effects are precluded.

*Characterization of the cholinergic receptors.* We attempted to identify the cholinergic receptors responsible for the effects of ACh. High doses of ACh caused a transient slight stimulation of the chemoreceptors, an effect which preceded the inhibition. This excitation appeared to be attributable to a *nicotinic* action of ACh since it was abolished by mecamylamine. We are unable to explain why the nicotinic agonist SDCh was inconsistent in its ability to stimulate the chemoreceptors. In any event, as the excitatory effect was slight and only seen following injection of high doses of ACh, it is unlikely to have much physiological significance.

The inhibitory action of ACh was evidently mediated by a *muscarinic* mechanism because the muscarinic agonist bethanechol, but not the nicotinic SDCh, also caused inhibition, and the response could be blocked by the muscarinic antagonist atropine, although relatively high doses were needed. Interpretation of data derived from experiments involving the use of atropine in rabbits is complicated by the presence of an atropinase enzyme in some rabbits (Ambache, 1955), a fact which may account for the large variation in the dose of atropine required to prevent the vascular effects of ACh. Nevertheless, it was possible to block the vasodepressor action of ACh with doses of atropine which were lower than those required to block the chemoreceptor inhibitory response.

The possibility exists that the muscarinic receptors mediating the inhibition of chemosensory activity evoked by ACh are 'atypical', perhaps being similar to those in the adrenal medulla (Henderson & Ungar, 1977), or in sympathetic ganglia (Hilton, 1977), which are also fairly resistant to atropine blockade. Further studies are required to investigate this possibility.

*ACh-dopamine interaction.* It has been suggested that ACh may release dopamine from the rat carotid body via a muscarinic mechanism (Hellström, Hanbauer & Costa, 1976). Since we found that dopamine inhibited rabbit chemosensory activity, and it is known to be present in the rabbit carotid body (Dearnaley *et al.* 1968), the possibility that ACh-induced inhibition was secondary to dopamine release became



attractive. However, it is unlikely to be the case because the dopamine antagonist  $\alpha$ -flupenthixol, in doses sufficient to block the inhibitory response to exogenous dopamine, did not reduce the inhibitory response evoked by ACh.

Although we found that dopamine inhibited carotid chemoreceptor activity in the rabbit, as it does in the cat (Docherty & McQueen, 1978a), it should be noted that Monti-Bloch & Eyzaguirre (1977) reported that it *increases* chemoreceptor discharge of the superfused rabbit carotid body *in vitro*. We found there was a delayed excitation following some doses of dopamine, but this effect was invariably preceded by an inhibition of chemosensory activity. A possible explanation for the difference between the *in vivo* and the *in vitro* results is that vascular effects are responsible for the inhibition observed *in vivo*; such vascular changes should not occur in the superfused preparation. Sampson, Aminoff, Jaffe & Vidruk (1976a) presented evidence showing that vascular effects are unlikely to account for the dopamine-induced inhibition of chemosensory activity in cats; whether a similar situation pertains in the rabbit remains to be established. However, we consider it unlikely that the inhibition observed *in vivo* is secondary to vascular effects of dopamine, particularly since the latency to onset of effect following the start of an injection is so short (1–2 sec), although we cannot entirely preclude the possibility. The discrepancy between the results seems more likely to be attributable to variability of the *in vitro* preparation's responsiveness to dopamine (Zapata, 1975) or to differences in dose used and the time during which the drug is present in the carotid body. The delayed excitatory effect observed *in vivo* may be mediated by a mechanism which perhaps involves a different type of dopamine receptor, but further studies are required to characterize this response.

Dopamine appears to be involved in modulating chemosensory activity in the cat (Mitchell & McDonald, 1975; Zapata, 1975; Docherty & McQueen, 1978a) and it is tempting to speculate that it has a similar role in the rabbit carotid body. However, this remains to be established.

*Physiological significance.* It is difficult to reconcile the present finding that ACh inhibits rabbit carotid chemosensory activity with the hypothesis that ACh is an *excitatory* transmitter in the chemosensory mechanism (e.g. see reviews by Heymans & Neil, 1958; Eyzaguirre & Zapata, 1968; Torrance, 1968; Biscoe, 1971; Howe & Neil, 1972). The nicotinic action of ACh in exciting sensory nerve endings may be non-specific, that is a direct action on the nerve endings which can be blocked pharmacologically without affecting the response of the endings to physiological stimuli (Brown & Gray, 1948; Gray & Diamond, 1957). It could be that whereas non-specific excitation of chemosensory afferents is observed in the cat and dog, it is not obtained, except transiently following high doses of ACh, in the rabbit. Could the threshold for non-specific excitation by ACh be higher in the rabbit than in other species? Is this related to levels of acetylcholinesterase? One could start speculating as to why inhibition of chemosensory activity occurs in rabbits, but it would be more profitable to study further the properties of the rabbit carotid body and sinus nerve and to compare them with those of the cat and dog (e.g. Verna's (1975) ultrastructural study of the rabbit carotid body).

It may well be that the inhibition evoked by ACh in rabbits is either indirectly mediated or else non-specific and lacking physiological significance, but it seems

worth exploring the possibility that these findings with exogenous ACh in rabbits, free from any masking excitatory effect of the drug, provide a clue regarding the physiological role of ACh in the carotid body. There is evidence for an efferent pathway running in the sinus nerve to the cat carotid body (e.g. see Biscoe & Sampson, 1968) and stimulation of this pathway inhibits carotid chemosensory activity (Neil & O'Regan, 1969; Belmonte & Eyzaguirre, 1974). Several workers have reported that this inhibition can be reduced by atropine (Willshaw, 1975; Goodman, 1975; Sampson, Aminoff, Jaffe & Vidruk, 1976b), although opinions differ about the mechanism of the inhibition and the physiological significance of the pathway (McCloskey, 1975). It remains to be established whether the rabbit carotid body receives an efferent innervation via the sinus nerve, but the possibility exists that the inhibitory effect of ACh on chemosensory activity in rabbits might result from an action on receptors involved in an efferent pathway. In such a scheme ACh would be a *modulator* or *regulator* of chemosensory activity rather than an excitatory neurotransmitter.

We are grateful to K. Bell and I. Maben for technical assistance and to Lundbeck & Co. for kindly providing the  $\alpha$ -flupenthixol. R. J. Docherty is in receipt of an M.R.C. research studentship.

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# The effects of benztropine and pargyline on the response of cat carotid chemoreceptors to sodium cyanide, acetylcholine and dopamine

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In the pentobarbitone-anaesthetized cat, exogenous dopamine causes inhibition of carotid chemoreceptor activity sometimes followed by a period of stimulation and it has been suggested that endogenous dopamine may modulate chemoreceptor activity (Docherty & McQueen, 1978).

In the present study the effects of benztropine, a dopamine uptake blocker, and pargyline, a monoamine oxidase inhibitor, on the chemoreceptor response to NaCN, ACh and dopamine were investigated.

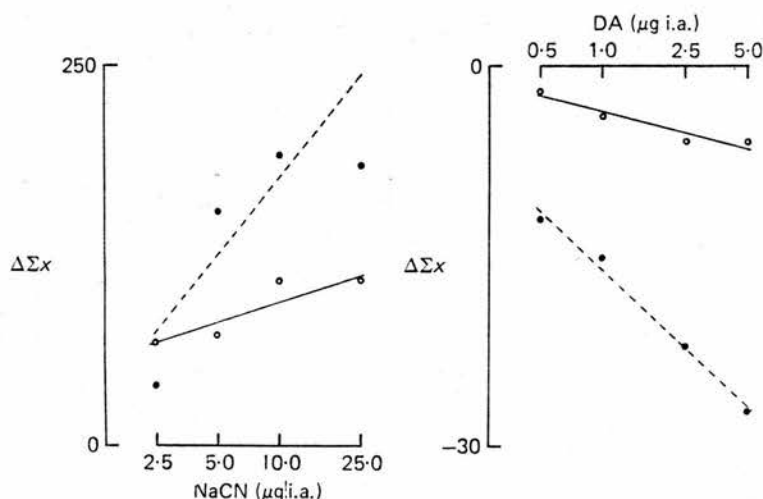


Fig. 1. Dose-response data obtained from a single chemoreceptor unit for NaCN and dopamine (DA) before (○—○) and after (●—●) administration of benztropine (0.25 mg/kg i.a.). Doses are plotted on a log<sub>10</sub> scale.

Chemoreceptor activity was recorded and quantified as previously described (McQueen, 1977) and drugs injected into the ipsilateral carotid artery. Benztropine (0.25–0.50 mg/kg i.a.) and pargyline (2.5–5.0 mg/kg i.a.) both augmented dopamine-induced inhibition of chemoreceptor activity and potentiated responses to NaCN (Fig. 1), benztropine being more effective than pargyline. Responses to ACh were unchanged by pargyline or benztropine.

It is difficult to estimate the influence of non-specific actions of benztropine or pargyline in these preliminary experiments, but the finding that drugs which augment the inhibitory effects of exogenous dopamine potentiate the response to NaCN suggests that endogenous dopamine may have an excitatory effect on carotid chemosensory activity in the cat.

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